Appendix A
Indicator Evaluation Criteria

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Individual Species Toxicity Tests Indicator:

How clearly is the proposed indicator linked to one or more of the sensitive Beneficial Uses?

> This indicator is a direct measure of the integrity of the aquatic community that either provides the Beneficial Use (Shellfish Harvesting, Commercial/Sport Fishing, Fish Migration, Fish Spawning) or are the entities the Beneficial Use is designed to protect (Wildlife Habitat, Estuarine Habitat). This indicator uses toxicity test results that were obtained from tests "clean" laboratory water and have the potential to overestimate the amount of toxicity present in ambient water.

How strongly linked is the indicator to potential effects of copper and nickel?

> This indicator is directly linked to the effects of copper and nickel through individual laboratory toxicity tests that measure the sensitivity of aquatic organisms to copper and nickel. This indicator facilitates the evaluation of the effects of copper and nickel in the Lower San Francisco Bay by providing required information to other indicators (AERAP and Site-Specific Studies).

What other stressors does the indicator respond to?

> This indicator measures the response of aquatic organisms to copper and nickel. For these tests, each metal is added singly to laboratory water that contains no other toxicants. This can be viewed as both a strength and weakness in that it isolates the effects of copper and nickel individually, but cannot distinguish individual effects when multiple stressors are present.

Does the proposed indicator provide an accurate representation of environmental conditions?

> This indicator provides the baseline for toxicity of copper and nickel to aquatic organisms and is considered to be a very conservative estimate of the effects of copper and nickel in ambient water. These tests are performed in a testing matrix that contains little or none of the constituents that compose the apparent complexing capacity of ambient water. This means that most, if not all, of the measured copper and nickel in the test solution is assumed to be available and toxic. In addition, this indicator does not account for the presence of other toxicants that may be present in ambient conditions.

Does the indicator communicate with Initiative TMDL stakeholders?

> This indicator provides very straight forward and easy to understand endpoints. These endpoints are survival, growth, or reproductive success in aquatic organisms. This allows the stakeholders to wade through the complexities of chemical-physical based indicators and ask the simple question, "could you live and reproduce under these conditions?"

Does the indicator have broad scientific acceptance?

> This indicator has broad scientific acceptance and use. It has been used to set national water quality criteria for both copper and nickel as well as for several other toxicants. In addition, it has been used as a base against which local water quality objectives have been compared and set.

Is this indicator measurable in the Lower South San Francisco Bay?

> There are currently several species and genera represented in the national data-set that currently reside in the Lower San Francisco Bay. It is desirable that additional resident species be added to the national data-set so that water quality objectives can be set that more adequately represent local water quality conditions. These species can easily be added to the national data-set by performing additional toxicity testing.

Is the indicator easy to use and inexpensive?

> The methods that are used for this indicator are well known, accepted by the scientific and regulatory communities, and relatively easy to perform. Since the bioassay field is very competitive, the costs to develop new/additional data-sets would be relatively inexpensive.

Are there adequate information available to support the use of the indicator?

> There are adequate data to provide a water quality criterion for both copper and nickel in marine systems. There are, however, fewer data for species that are resident to the Lower South San Francisco Bay. This indicator would provide a better estimate of local impairment if it contained a larger quantity of sensitivity data on the effects of copper and nickel to resident species.

Can the indicator be used in combination with other indicators?

> This indicator should be used only in conjunction with the AERAP and Site-Specific Indicators. Used alone, it tends to produce criteria that are over-protective of beneficial uses. Used with the AERAP and Site-Specific Indicators, it can provide a much more accurate estimation of whether there is any local impairment being caused by copper and nickel.

What are the uncertainties associated with the use of this indicator?

> How well does a laboratory toxicity response mirror the toxicity response observed in the field? How do water quality criteria developed in clean water represent ambient conditions? How well do surrogate species represent resident species? Do the species in the national data-set provide adequate protection for resident species?

AERAP Indicator:

How clearly is the proposed indicator linked to one or more of the sensitive Beneficial Uses?

> The status of community taxa is an essential element of most Beneficial Uses. This indicator is a direct measure of the integrity of the aquatic community that either provides the Beneficial Use (Sports and Commercial Fishing) or are the entities the Beneficial Use is designed to protect (Estuarine Habitat). The unique feature of this indicator is rather than measuring the well being of a single organism or species, stakeholders can evaluate overall aquatic community health.

How strongly linked is the indicator to potential effects of copper and nickel?

> The indicator is directly linked to the potential effects of copper through individual laboratory toxicity tests that measure the sensitivity of resident organisms to dissolved copper. The indicator facilitates evaluation of the effects of copper on community structure and function. That is, are all primary producers at risk from ambient concentrations of copper? The indicator could be linked to nickel in the same manner if more toxicity tests were available for nickel.

What other stressors does the indicator respond to?

> The indicator predicts the response of community taxa to measured and proposed levels of copper. The AERAP does not account for other stressors that may be acting on community taxa such as exotic species, physical habitat loss and degradation, and other pollutants. It can be viewed as both a strength and weakness of the AERAP that it isolates the effect of copper on community taxa.

Does the proposed indicator provide an accurate representation of environmental conditions?

> The AERAP provides a method to evaluate the impacts of copper t the ecologically meaningful level of community taxa. However, it is important to keep in mind a few aspects of the AERAP that cause it to fall short of a complete representation of environmental conditions. The AERAP relies on laboratory toxicity tests to estimate the impacts of copper on community taxa. Therefore, the indicator has the same caveats and assumptions as those for individual laboratory

toxicity tests. This includes the use of a testing matrix without any of the constituents that compose the apparent binding capacity of ambient water. In addition, the AERAP does not account for other stressors that may also be acting on community taxa. The AERAP is not dynamic. It cannot evaluate the ability or inability of local populations to respond or rebound from exposures to copper.

Does the indicator communicate with Initiative TMDL stakeholders?

The indicator uses statistical methods that many stakeholders may be unfamiliar or have little experience with. However, the model output is an easily understood measure of environmental conditions and is directly linked to the recommendation (e.g., SSO) that the stakeholder group will be making. The indicator is supported by strong graphical representation of results that ease the interpretation of the AERAP. The indicator is a flexible tool that can be used by stakeholders to evaluate a wide range of conditions.

Does the indicator have broad scientific acceptance?

The indicator was developed through a peer review process sponsored by the Water Environment Research Foundation (Parkhurst et al 1996). It has been used by regulatory agencies as a technical tool for determining cleanup levels, assessing impacts and setting pollutant control program priorities, and in the development of site-specific water quality objectives. The method is cited in the U.S. EPA "Guidelines for Ecological Risk Assessment" (U.S. EPA 1998). Guidelines

Is the indicator measurable in the South Bay?

The indicator requires the use of a toxicity effects database for resident species. The project team was able to compile an adequate amount of information on the sensitivity of resident species to copper. This included 26 species representing a wide range f ecological niches and sensitivities. The project team was unable to obtain an adequate number of toxicity tests for species measuring their sensitivity to nickel. The indicator was not applied for nickel.

Is the indicator easy to use and inexpensive?

The difficult aspect of using this indicator is acquiring water quality monitoring data and species toxicity tests for the pollutants to be considered. The toxicity tests for nickel would be routine, but would require approximately six months and an estimated $25,000 to produce the necessary database. The AERAP software is widely available from the Water Environment Research Foundation. The software comes with documentation that would allow most stakeholders to perform the analyses on most computers. The output can be printed to most printers. The WERF design requirements for the AERAP were for easy access to provide most stakeholders to have the opportunity to directly perform their own

risk evaluations. The project team will instruct any interested stakeholders in the use of the AERAP software.

Is there adequate information available to support the use of the indicator?

As noted earlier there is adequate ecological effects characterization for resident species for copper but not for nickel. The City of San Jose South Bay Monitoring Study and the RMP adequately characterize the expected environmental concentrations of dissolved copper and nickel.

Can the indicator be used in combination with other indicators?

The indicator should be used in combination with site-specific studies and plankton to complete the analysis and, to further consider uncertainties associated with the indicator. Site-specific studies provide the basis for extrapolating the laboratory toxicity tests results to the ambient environment. Plankton provides information for further consideration of the selection of the ERC level.

What are the uncertainties associated with the use of this indicator?

How completely has the aquatic community been characterized in the resident species toxicity database? How well have ambient exposure patterns been characterized? How important is the potentially impacted taxa to maintaining ecosystem integrity and sustaining designated Beneficial Uses.

Site-Specific Studies Indicator:

How clearly is the proposed indicator linked to one or more of the sensitive Beneficial Uses?

This indicator is a direct measure of the integrity of the aquatic community that either provides the Beneficial Use (Shellfish Harvesting, Commercial/Sport Fishing, Fish Migration, Fish Spawning) or are the entities the Beneficial Use is designed to protect (Wildlife Habitat, Estuarine Habitat). This indicator provides a more accurate estimate of ambient conditions since it includes the use of ambient site-water and/or resident species.

How strongly linked is the indicator to potential effects of copper and nickel?

This indicator is directly linked to the effects of copper and nickel through individual laboratory toxicity tests that measure the sensitivity of aquatic organisms to copper and nickel. This indicator provides a measure of the maximum allowable concentrations of copper or nickel that can be present in the Lower South San Francisco Bay without impairing beneficial uses.

The response of aquatic organisms in copper and nickel-spiked Lower South San Francisco Bay site water is a direct laboratory assay of the effects of copper and nickel in the field. It accounts for any additive, competitive, or synergistic effects of copper and nickel with other potential toxicants present in the (site) water. Thus, it is strongly linked to potential effects of copper and nickel in the field.

What other stressors does the indicator respond to?

This indicator responds to everything that is present in the Lower South San Francisco Bay site waters that is bioavailable to aquatic organisms. This indicator is a measurement of the response of aquatic organisms to copper and nickel in actual site water and therefore takes into account any additive or synergistic effects of copper and nickel with other potential toxicants present in the (site) water at the time of collection. Other aspects of this indicator include using resident species sensitivities to copper and nickel to provide a better estimate of ambient water quality conditions.

Does the proposed indicator provide an accurate representation of environmental conditions?

This indicator provides a direct assay of the amounts of copper and nickel that are bioavailable to the most sensitive species in the data-set. It provides an accurate representation of environmental conditions *in the water column*.

Does the indicator communicate with Initiative TMDL stakeholders?

This indicator provides very straight forward and easy to understand endpoints. These endpoints are survival, growth, or reproductive success in aquatic organisms. This allows the stakeholders to wade through the complexities of chemical-physical based indicators and ask the simple question, "could you live and reproduce under these conditions?"

This indicator represents "good science" and "data driven" decision making, two concepts with which most stakeholders will identify.

Does the indicator have broad scientific acceptance?

This indicator has broad scientific acceptance and use. It has been used to set national water quality criteria for both copper and nickel as well as for several other toxicants.

This indicator has been used most recently by the City of San Jose to provide a basis against which a local water quality objective could be set. A preliminary review of this study by EPA (Dr. Glen Thursby) concerning the appropriateness of the methodology, the quality of the data, and the reasonableness of the conclusions was very favorable. Also, the EPA (Prothro 1993) officially

concluded and recommended that dissolved metal be used to set and measure compliance with Water Quality Standards since dissolved metal more closely approximates the bioavailable fraction of the metal in the water column than does total recoverable metal. This conclusion was supported by a majority of the scientific community, both within and outside of the EPA (Prothro 1993).

Is this indicator measurable in the Lower South San Francisco Bay?

This indicator is measurable in South Bay. A water-effect ratio can be determined for any station location at any time during the year (wet or dry season) or for any tidal cycle, depth, etc. The water-effect ratio (WER) is the key component of the indicator. The product of the WER and the national criterion is the site-specific criterion. It is the derived site-specific criterion that should not be exceeded in order to protect beneficial uses at the site.

Is the indicator easy to use and inexpensive?

This indicator requires considerable expertise and expense. The city of San Jose has provided an unprecedented database from which the current WER values and suggested site-specific criteria (objectives) were derived. Periodic confirmation of WER values may be necessary. Since the WER values link the *Mytilus* response in copper-spiked South Bay site water to the site-specific criterion, routine metals chemistry monitoring (as is now done by RMP) may be sufficient to ensure that the site-specific criterion value is not exceeded.

Are there adequate information available to support the use of the indicator?

The final Water-Effect Ratio (FWER) used to derive the suggested site-specific copper criterion is based on a large (unprecedented nationally) database (n=40). These WERs were chosen from an even larger pool of derived WERs (n=134). San Mateo, Coyote Creek, and total copper WERs for all stations were not used to derive the FWER. Analysis of the WER data as well as the associated ambient copper and TSS values supports the use of a dissolved copper criterion to protect water quality in the South Bay.

The EPA WER methodology has undergone significant improvements in the past 15 years. The understanding of metals chemistry as applied to WERs has also undergone significant, recent change. The aspects of this "new" understanding that are most pertinent to the choice of *Mytilus* as an indicator of copper impairment in the South Bay are:

- Dissolved metal more closely approximates the bioavailable fraction of metal in the water column and

- Species whose sensitivities are near to but above the criterion for a metal (e.g., *Mytilus*, copper) are the most appropriate for use in determining site-specific criteria (WERs) since they best estimate the bioavailability of metal at the criterion concentration.

There is a body of data to draw upon to establish protective levels of nickel in marine water. Two species in the City of San Jose's nickel ACR study are among the lowest values in the dataset. The new acute data for the red abalone sets the FAV and CMC. Also, the study added three new chronic numbers to the dataset, there had previously been only one marine chronic value. There are now four potentially valid marine ACRs on which to base a marine FACR.

There is also a growing database of measured total and dissolved nickel in San Francisco Bay upon which to base appropriate site-specific criteria.

Can the indicator be used in combination with other indicators?

This indicator should be used in conjunction with the AERAP and Individual species toxicity test indicator. Used with the AERAP and Individual species toxicity test indicators, it can provide a much more accurate estimation of whether there is any local impairment being caused by copper and nickel.

What are the uncertainties associated with the use of this indicator?

How well does a laboratory toxicity response mirror the toxicity response observed in the field? How well do surrogate species represent resident species? Do the species in the national data-set provide adequate protection for resident species?

Phytoplankton Indicator:

How clearly is the proposed indicator linked to one or more of the sensitive Beneficial Uses?

This indicator forms the base of the food-chain and is an essential component of all sensitive beneficial uses. This indicator is a direct measure of the integrity of the aquatic community that either provides the Beneficial Use (Shellfish Harvesting, Commercial/Sport Fishing, Fish Migration, Fish Spawning) or are the entities the Beneficial Use is designed to protect (Wildlife Habitat, Estuarine Habitat).

How strongly linked is the indicator to potential effects of copper and nickel?

Phytoplankton are among the most sentitive organisms to copper and nickel. This indicator has been directly linked to the effects of copper and nickel through individual laboratory toxicity tests that measure the sensitivity of phytoplankton to copper and nickel.

What other stressors does the indicator respond to?

The presence and distribution of phytoplanktonic organisms is influenced by several other environmental conditions, including:

- Physical,
- Chemical, and
- Biological.

Therefore, it is critical to carefully consider the ambient environmental conditions of the site when using this indicator.

Does the proposed indicator provide an accurate representation of environmental conditions?

Phytoplanktonic assemblages provide an indication of the health of the phytoplanktonic community (e.g., a larger number of sensitive species vs. non-sensitive species) and, as such, the health of the bay.

The phytoplankton form the base of the food-chain and provide a fundamental indicator of the ability of the Bay to sustain fish and other animals. The South Bay phytoplankton assemblages were responsible for over 60% of the primary production in San Francisco Bay in 1993.

Does the indicator communicate with Initiative TMDL stakeholders?

The fact that the phytoplankton form the base of the food-chain and provides a fundamental indicator of the ability of the Bay to sustain fish and other animals, is one that stakeholders can easily recognize.

Does the indicator have broad scientific acceptance?

The use of community structure indices have been widely used by environmental scientists. However, the Lower South San Francisco Bay phytoplankton population structure and dynamics, while being important to the health of the Bay, has not been adequately characterized.

Is this indicator measurable in the Lower South San Francisco Bay?

> This indicator is an existing component of the USGS studies in the Lower South San Francisco Bay. However, there have only been a few studies performed and the dataset does not contain information on all phytoplanktonic size classes. In addition, there is a lack of adequate temporal data.

Is the indicator easy to use and inexpensive?

> Developing the indices would be very expensive and time consumptive. Researchers are currently just beginning to understand the community structure of the South Bay phytoplankton population.

Are there adequate information available to support the use of the indicator?

> The USGS has been monitoring the Lower South San Francisco Bay phytoplankton population and have some information regarding community structure. However, this information is limited in scope and is not adequate to characterize the conditions of the phytoplankton populations within the Bay.

Can the indicator be used in combination with other indicators?

> This indicator can only be used qualitatively and as a comparative benchmark against which the other indicators can be compared.

What are the uncertainties associated with the use of this indicator?

> What role does metal speciation (free or dissolved) play in any observed toxicity? How does the production of phytochelators affect metal toxicity? What are the effects of sample handling on metal toxicity? What is the composition of the Lower South San Francisco Bay phytoplankton population?

Appendix B
Palo Alto Clam Study

USGS
science for a changing world

Bioaccumulation of metals by the bivalve *Macoma balthica* at a site in South San Francisco Bay between 1977 and 1997: Long-term trends and associated biological effects with changing pollutant loadings.

U. S. Geological Survey

OPEN FILE REPORT 99-55

Prepared in cooperation with
CITY OF PALO ALTO, CALIFORNIA

Bioaccumulation of metals by the bivalve *Macoma balthica* at a site in South San Francisco Bay between 1977 and 1997: Long-term trends and associated biological effects with changing pollutant loadings.

By Michelle Hornberger, Samuel Luoma, Daniel Cain, Francis Parchaso, Cynthia Brown, Robin Bouse, Christopher Wellise, Janet Thompson.

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Menlo Park, California

U. S. DEPARTMENT OF THE INTERIOR

BRUCE BABBITT, Secretary

U. S. GEOLOGICAL SURVEY

Charles G. Groat, Director

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Bioaccumulation of metals by the bivalve *Macoma balthica* at a site in South San Francisco Bay between 1977 and 1997: Long-term trends and associated biological effects with changing pollutant loadings.

By Michelle Hornberger, Samuel Luoma, Daniel Cain, Francis Parchaso, Cynthia Brown, Robin Bouse, Christopher Wellise, Janet Thompson.

ABSTRACT

Although waste water discharge into San Francisco Bay has increased since the 1950's, this has been accompanied by investments in advanced treatment. Since 1976, near-monthly samples of sediments and the deposit feeding clam, *Macoma balthica*, have been collected near the Palo Alto Regional Water Quality Control Plant (PARWQCP) to determine how changes in waste water discharge affected trends in silver, copper and zinc concentrations. Long-term reductions of metals in *M. balthica* and sediments were evident as waste water treatment improved at PARWQCP. Mean annual silver concentrations in *M. balthica* were 106 μg/g in 1978, 55.4 μg/g in 1987 and 3.6 μg/g in 1997. These declines coincided with improved treatment processes implemented by PARWQCP. Mean annual concentrations of copper in *M. balthica* declined from a maximum of 287 μg/g in 1980 to the minimum of 24 μg/g in 1991. Temporal changes in zinc concentrations were nearly bimodal, with the highest concentrations occurring during the years of high precipitation. Copper bioaccumulation was strongly correlated with copper loads from the PARWQCP until 1990, suggesting that effluents from the treatment plant were the primary source of copper to *M. balthica* during this period. Copper loadings from the PARWQCP continued to decline steadily after 1990, but copper in *M. balthica* continued to increase to a high of 71 μg/g in 1996, showing no significant correlation to copper loadings. Thus, as the localized sources of copper decreased, inputs from outside sources (for example, urban runoff), became more important in controlling metal bioaccumulation. Stream flow and precipitation were used as surrogate measures for metal loads entering into the Bay and show a strong

correlation to copper bioaccumulation from 1989-97. The high concentrations of metals in the mudflat may have had an adverse affect on the resident population of organisms as measured by the low number of reproductively active individuals present between 1974-1983 (<20 percent). As metal concentrations began to decrease, 70-100 percent of the population were reproductively active. Reproductive patterns typical of less impacted sites in the Bay were not consistently observed at the Palo Alto mudflat until 1989, corresponding to mean annual copper concentrations of 35 μg/g in tissues and mean annual silver concentrations of 11 μg/g.

INTRODUCTION

The importance of understanding contamination trends is well recognized by local, state and federal agencies responsible for maintaining the water quality of San Francisco Bay (Monroe and Kelly, 1992) and by the national and international scientific and regulatory community (Tanabe and others, 1989; Daskalakis and O'Connor, 1995). As in many estuaries (Nixon, 1995), historical waste discharges into San Francisco Bay are not fully known. It is known that the volume of municipal waste discharge has grown. Available estimates suggest that discharges to the Bay overall were 230 million gal/d in 1955 and 555 million gal/d in 1986 (Monroe and Kelly, 1992). Implementation of advanced waste water treatment has accompanied the growth in discharge rates, especially since the passage of the Clean Water Act in 1972. The net effect has been reduced contaminant loadings. Data reported by municipal dischargers to San Francisco Bay indicate that total metal loadings declined from 993 T/yr in 1960 to 171 T/yr in 1986 (Monroe and Kelly, 1992). The responses of the ecosystem to these reductions in metal loadings are not fully known. Systematic monitoring of contaminants has occurred in the Bay, at the regional scale, since 1993 (San Francisco Estuary Institute, 1994, 1995, 1996). However, this database does not include the years of greatest loadings into South San Francisco Bay. These regional trends are a reflection of the combination of trends from a large number of local contaminant inputs (Luoma and Cloern, 1982; Luoma and Phillips, 1988), combined with the influences of physical processes that drive mixing within the estuary (Ritson and others, in press). Monitoring of trends near local sources of contamination may provide insights unavailable from regional monitoring. The data presented here are unique because the period of collections encompasses a temporal scale of high metal discharge followed by reductions. Because of the long duration of the near-monthly sampling, the response of bioaccumulation at a local scale can be measured against effects from a larger ecosystem response.

Sediments and benthic organisms are commonly used as indicators to determine spatial distributions and temporal trends of trace metal contamination in aquatic

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environments (Phillips, 1980; Phillips and Rainbow, 1993). Most metals bind strongly to fine-grained (silt/clay) sediments and retain a record of metal release to an environment (Luoma and others, 1990). Analysis of the tissues of organisms complements sediment analyses and can be an effective means of estimating trends in bioavailable metal exposures. Organisms may even be more sensitive indicators of anthropogenic metal inputs than sediments. Different species concentrate metals to different degrees, but if one species is analyzed consistently over time, the results can be successfully employed to indicate trace element exposures to the food web (Phillips and Rainbow, 1993).

In the present study, we show that both fine-grained sediments and an indicator organism (the bivalve *Macoma balthica*) are effective monitors of long-term trends, sources and effects of metal contamination in South San Francisco Bay. Since 1977 USGS personnel have monitored and studied trace metal concentrations in sediments and the resident bivalve, *Macoma balthica*, in the vicinity of the discharge of the Palo Alto Regional Water Quality Control Plant (PARWQCP) (Luoma and others, 1985; 1991; 1992; 1993; 1995a; 1996; 1997; 1998). This is an unusually detailed data set in that samples were collected on a near-monthly basis for twenty years. The frequent sampling allows assessment of trends on multiple time scales (seasonal, annual and decadal) as the PARWQCP changed and improved its treatment methodologies. The site location, study design and ancillary data collection also allow consideration of environmental influences within the zone of influence of the PARWQCP. Environmental factors may or may not confound water quality trends, but their influence must be considered (Hem, 1991). The primary objective of this report is to describe the long-term (20 year) trends in metal concentrations in sediments and *M. balthica*; to determine if the influence of factors other than the local point source have an effect on trends; and to evaluate biological responses to metal contaminants. We demonstrate that concentrations of the metals that comprised the most significant water quality problems near the PARWQCP declined between 1977 and approximately 1989-91, but concentrations have stabilized during the last decade. The primary source of the

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contamination seems to have shifted from the PARWQCP to other sources whose inputs appear to be positively correlated with surrogate measures for urban runoff (precipitation and freshwater discharge). Reconstruction of reproductive capability from archived specimens, and comparisons with earlier studies provide evidence that the contamination at the Palo Alto mudflat prevented successful reproduction in this species when the contamination was most severe.

Study Site

The PARWQCP discharges treated effluent into a marsh channel about 1 km upstream from where the channel empties into the intertidal zone of South Bay. The monitoring site is located on an intertidal mudflat 1 km south of the channel mouth (fig. 1). Thomson and others (1984) identified the PARWQCP as the principle source of silver and copper to intertidal mudflats south of the discharge channel.

The hydrologic and hydrographic characteristics of the receiving waters are an important consideration determining the fate and biological availability of the constituents of effluents entering South Bay (Luoma and Cloern, 1982; Thomson-Becker and Luoma, 1985; Luoma and others, 1985; Hostettler and others, 1989). South Bay is a large coastal embayment with freshwater inputs from the Sacramento/San Joaquin River system, local stream inflows and wastewater discharges. River inflows from North San Francisco Bay penetrate South Bay during only the years of high winter - spring runoff, when river inflows exceed 40,000 ft³/s. Urban development dominates the lower watershed of all local streams that enter the South Bay. Most urban runoff is collected in storm drains and discharged into local creeks. However, most streams are constrained above the urban watershed by small reservoirs. Most stream gages in this system are located above the reservoirs, making it difficult to adequately estimate the total amount of freshwater inputs into the South Bay.

Local stream flow and river inflows, in response to precipitation, are characterized by strong seasonal and interannual variability (Conomos, 1979) (fig. 2).

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Annual fluctuations are driven by a wet season (winter - spring precipitation) and a dry summer - autumn season when precipitation stops. Most local stream inflow to the Bay stops entirely during the dry season, and wastewater becomes the predominant source of freshwater during summer and fall at the Palo Alto site.

Salinity changes in the South Bay are influenced by local stream inflows, as well as periodic high inflows from the Sacramento and San Joaquin Rivers in North Bay. Reduced salinities during the wet season of the year are evident and follow the general trend of freshwater inputs during years of highest rainfall and snowmelt (fig. 2). Salinity can directly affect the speciation and bioavailability of some metals (Sunda and others, 1978; Nugegoda and Rainbow, 1989; Luoma and others, 1990).

Sediment characteristics also are affected by the hydrologic characteristics of the South Bay ecosystem (Thomson-Becker and Luoma, 1985). South Bay has large shallow expanses of less than 2m deep (Conomos, 1979), and strong diurnal winds occur most of the year, although wind velocity has a consistent seasonal pattern and is at a maximum from June through August. Sediments are mixed and resuspended from the shallows by wind and tidal currents, especially through the summer when velocities are highest (Schoellhamer, 1996). Runoff from local streams and the larger rivers replenish the fine-grained (silt/clay) sediments annually during the high inflow season. Because of the yearly renewal and resuspension of sediments, continual deposition is not characteristic of the intertidal zone in South Bay (Fuller, 1982). A pulse of pollutant inputs to a mudflat is probably at least partly dispersed each year and mixed through the Bay (Luoma and others, 1997) and the mudflat is replenished with terrigenous material annually (Thomson-Becker and Luoma, 1985).

METHODS

Near-monthly samples of surface sediment and the deposit feeding clam, *M. balthica* were collected at the Palo Alto mudflat between 1977 and 1997 (fig. 1). Surficial sediment samples were collected by gently scraping the oxidized layer (approximately 1-2 cm) from the surface. Samples were placed in a clean plastic container, transported to

the laboratory, and wet-sieved through a trace-metal clean 100μm polyethylene mesh with ultra pure deionized water. A mesh size of 100μm was used because it represents the largest sized particles that are digested by *M. balthica* (Luoma and Cain, 1979). Furthermore, because of the seasonal variability in grain size that occurs within a year, sieving eliminates the bias that might occur as a result of coarser particles that accumulate during the summer months (Luoma and others, 1997).

Sand/silt ratios were determined for all samples collected since 1992, using a method described by Hornberger and others, (in press). The fraction of sample that did not pass through the 100μm sieve was placed into a tared petri dish and dried at room temperature. The <100μm fraction was covered with a watch glass and dried at 60°C. Samples were homogenized using a mortar and pestle, split into 0.5g replicate aliquots, and placed in a tared 22 mL scintillation vial. Each sample was redried and weighed to the nearest milligram. Total Organic Carbon was measured using a carbon analyzer on sieved sediment by methods outlined by Thomson-Becker and Luoma (1985).

Sediment metal concentrations were determined by two chemical extraction methods: a near-total digest and a two hour extraction with weak acid (0.6 N HCl). Samples analyzed using the near-total digest were refluxed in 10 mL of concentrated HNO_3 at 150°C for approximately 2 weeks, or until the digest was clear. Although this method does not provide complete dissolution of all metals, it is indicative of metals sufficiently mobile to be of toxicological interest. It is also comparable to the method employed the Environmental Protection Agency and with procedures used by the San Francisco Bay Regional Monitoring Program (Hornberger and others, in press). A recent comparison across a spatial range of San Francisco Bay sediments showed that near total metal concentrations correlate strongly with metal concentrations determined after complete decomposition (Hornberger and others, in press). After decomposition, samples were reconstituted in 0.6N HCl and filtered through a 0.45 μm filter. The weak acid digest consisted of a two hour extraction with 0.6 N HCl. This extraction correlates well with silver bioavailability from sediments (Luoma and others, 1995b). Samples were filtered through a 0.45 μm filter after extraction and analyzed directly. All metal

concentrations are reported in μg/g dry weight. Concentrations of silver from sediments are reported since 1977 only for 0.6N HCl extractions.

The deposit feeding clam, *Macoma balthica*, was collected simultaneously with the sediment. At least 40 individuals of varying size ranges were collected. Animals were returned to the laboratory and allowed to depurate the content of their digestive tract for 48 hours (Luoma and Cain, 1979). Clams were sorted into separate size ranges, determined by differences of 1mm shell length. Individuals within each size class were composited for a single sample, and soft tissues were removed from the shells for analysis. Each sampling data consisted of approximately 6-13 composites, each composite, consisting of 3-7 animals of similar shell length. Animal tissues were dried, weighed and refluxed in concentrated HNO_3 until the digest was clear. Digests were evaporated to dryness, reconstituted in 0.6 N HCl and filtered through a 0.45 μm filter. All metal concentrations are reported in μg/g dry weight.

Different analytical techniques have been used for the analysis of sediments and clams. Between 1977-1989, copper and zinc in tissues and sediments were determined by flame atomic absorption spectroscopy (AAS) (Thomson and others, 1984). Since 1990, metals for both sediments and *M. balthica* have been determined by Inductively Coupled Argon Plasma Emission Spectroscopy (ICAPES). Selected archived sediment samples (1977-83) were re-analyzed by ICAPES to determine concentrations of chromium, nickel and vanadium. Cadmium and silver concentrations for all sediments were determined by Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS) with Zeeman background correction and standard additions technique. Mercury and selenium analysis were determined in both sediment and clam tissues by Hydride Generation Atomic Absorption Spectrophotometry. For mercury and selenium analyses, samples were digested at 100°C in aqua regia followed by 10 percent nitric/dichromate reconstitution; 3 percent $NaBH_4$ (in 1 percent NaOH) was added to the digestate as a reductant before analysis by cold vapor AAS.

Quality control was carefully maintained through the entire study period, including routine analysis of procedural blanks and the measurement percent recoveries of

Standard Reference Material (Luoma and others, 1997). All values were within the acceptable range of certified values.

Precipitation data were collected by the National Weather Service (NWS) at stations throughout San Francisco Bay. Precipitation into South San Francisco Bay was estimated by averaging the total amount measured in San Francisco and the total amount measured in San Jose. While the two stations followed similar trends within each year, the amount of total rainfall in San Francisco is slightly greater than San Jose. The Palo Alto study site lies nearly midway between the two cities and was represented by averaging the two end members. Total annual streamflow into South San Francisco Bay was measured using the sum of freshwater discharge from three creeks: Saratoga, San Francisquito and Guadalupe Creeks. These creeks were chosen as the best representation of measurable freshwater inputs in the South Bay (Schemel, USGS, oral commun., 1998). To provide a comparable scale to the bioaccumulation data, all discharge data are presented by calendar year.

Reproductive activity was determined using stained thin sections of the visceral mass of preserved specimens of *M. balthica* that had been collected from the Palo Alto mudflat between 1974 and 1989 using methods described for other bivalves (Parchaso and others, 1997). Because of the seasonal nature of the reproductive cycle (Nichols and Thompson, 1982), repeated sample collection throughout a year is essential for a valid evaluation of reproductive capabilities. Between 1974 and 1989, there were four periods when data was available from near-monthly collections: Feb. 1974 - July 1975; June 1979 - October 1981; January 1983 - February 1985; and December 1987 - September 1989. Only animals of reproductive age were collected for sectioning and, on most dates, 10 clams were examined. Each specimen was characterized by sex, developmental stage, and condition of gonads. Gonadal development was characterized as one of five stages: inactive, active, ripe, spawning, and spent. For the purposes of simplification in this report, reproductive capability was defined by the ability to reach reproductive maturity, and five stages were aggregated into two categories. Reproductively active animals ("percent reproductive") were defined as clams whose

gonads contained cells that were reproductively mature (stages "ripe," "spawning," and "spent"). The gonads of clams classified as non-reproductive had follicles that had not achieved reproductive maturity (inactive) or were in the initial stages of the development of reproductive activity. The latter stage indicates that animals might eventually reproduce, but had not reached maturity. Prolongation of the active phase of cell development and/or failure to mature beyond this stage can be a response to stress.

RESULTS

Trends in PARWQCP Inputs

The history of the PARWQCP is similar to that of other municipal dischargers in the San Francisco Bay area and the nation. Passage of the Clean Water Act in 1972 initiated improvements in wastewater treatment nationwide. The PARWQCP officially became a regional facility and secondary treatment became operational in October, 1972. Upgrades to treatment processes occurred in 1980 and 1988-89 (P. Bobel, Palo Alto Environmental Compliance Division Manager, written commun., 1998). In 1980, advanced treatment was added, including trickling filters (which allowed nitrification to occur in the pre-existing aeration tanks) and dual media filters. In 1988-89, clarifiers were added improving solids removal, especially during wet weather. Source control programs for silver and copper began in 1989 and have become a national model. In the mid 1990's, bacteria retention times were increased, and bar screens replaced barminuters.

The flow rate of the PARWQCP effluent has not changed greatly since the late 1970's, and has averaged $26.1 \times 10^6 \pm 3.8$ L/d (fig. 3). Unfiltered effluents have been analyzed by plant personnel since the late 1970's; copper and zinc probably offer the most reliable analytical data. Copper concentrations in the effluent as high as 150 ug/L were determined in the late 1970's and estimated total loadings reached a peak of approximately 5,800 kg/yr in 1979 (fig. 3). Concentrations of copper in 1996 averaged 5 ug/L and total annual loadings were 173 kg/yr. In sharp contrast, zinc concentrations have not changed in the effluent over time, nor have loadings changed. In 1996, mean

concentrations in effluent were 54 mg/L and loadings were 1,871 kg/yr.

Stream Inflows

During the present study, a combination of wet and dry years occurred; but there was no long-term unidirectional trend in precipitation or freshwater inflows. Pronounced periods of low precipitation and low inflows occurred in 1976-77, 1984-85, and from 1987 through 1991 (fig. 2). As a result of a strong ENSO (El Nino) event, 1983 was the wettest year of this century (before 1997-98) with some of the highest inputs of freshwater into South San Francisco Bay recorded during the study. Extreme rainfall in spring 1986 resulted in the highest recorded river flow in the century. Inflows that were high, but more typical of the long-term average, occurred in 1993, 1995 and 1996. Another strong ENSO occurred in 1997-98 which was accompanied by very high inflows.

Monthly salinity varied from 5 to 33 at the Palo Alto mudflat over the >20 year study period. Annual mean salinities followed a generally bimodal trend reflecting the droughts of 1976-77 and 1987-1992, interspersed with the high rainfall of the 1982-86 and 1993-1996 periods. Seasonal rainfall patterns controlled the seasonal cycle within years (fig. 2). The high rainfall years in 1982-83 and 1995-96 resulted in the lowest mean annual salinities and instances of the lowest monthly salinity in the 20 year period. In 1982-83, the mean annual salinity at the Palo Alto mudflat was 13.3 ± 2.2, about 30 percent less than average salinities for a more normal year (ie, 1979-80, 21.8 ± 2.3). The 1987 - 1992 drought was evidenced by salinities in excess of 22 throughout the period.

Geochemical stability of the Palo Alto mudflat

The geochemical nature of the sediment, indicated by Total Organic Carbon (TOC) and concentrations of iron and manganese showed no significant unidirectional, long-term trends on the mudflat. Four time segments were selected around the major implementations of wastewater treatment improvements. Total Organic Carbon (TOC) in

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the Palo Alto mudflat averaged 1.19 ± 0.38 percent dry weight between 1977 and 1997 (table 1). The full range of TOC over the study period was 0.4 - 2.1 percent dry weight. Seasonal variability exceeded year-to-year variability (see also Thomson-Becker and Luoma, 1985). The mean TOCs were not significantly different among the four time segments. The stability of the long-term data suggested that changes in TOC would not be responsible for long-term changes in metal concentrations.

Concentrations of near-total iron averaged 4.4 ± 1.2 percent dry weight between 1977 and 1997 (table 1). Manganese concentrations averaged 1074 ± 380 μg/g. Low annual mean concentrations of both iron and manganese occurred from 1983-85, when high precipitation, high stream inflows, and short residence times prevailed in South Bay (fig. 4). Conversely, during the 1987 - 1992 drought period, high manganese concentrations and slight increases in iron concentrations were evident. A trend of increasing annual mean and annual maximum iron concentrations (but not manganese) is evident in Palo Alto sediments since the mid-1980's (fig. 4). The year-to-year fluctuations of redox-sensitive iron and manganese in the oxidized surface sediments could be caused by a variety of factors. One possibility is that differences in hydraulic residence times in association with different freshwater inflows may affect flushing and the depth of the redox interface in the sediments. Nevertheless, geochemical factors that might affect metal sequestration by sediments or metal bioaccumulation by clams (Luoma and Bryan, 1979; Bachtiar, and others. 1996) were not progressively changing during the study period.

Concentrations of chromium, nickel and vanadium are naturally enriched in the San Francisco Bay watershed (Hornberger and others, in press) and can be used as an indicator of changing environmental conditions over time. Although mean concentrations of chromium, nickel and vanadium were slightly higher in 1990-97 than in the archived samples taken from 1977-83, they were not significantly different (fig. 5). The eight-year average for chromium concentrations, since 1990, was 116 ± 17 μg/g; the mean concentration in the 1977 - 83 samples was 98 ± 21 μg/g. Nickel concentrations in surface sediments collected since 1990 averaged 96 ± 11 μg/g; the mean for 20

samples from 1977 - 83 was 77 ± 14 μg/g. Vanadium concentrations averaged 66 ± 19 μg/g during the 1977-83 time period; the mean between 1990-96 was 94 ± 18 μg/g. All chromium, nickel and vanadium values from all periods were within the range typical of San Francisco Bay before human activities disturbed sediments (Hornberger and others, in press).

Metal Trends in Sediments

Silver concentrations in surface sediments decreased by three-fold between 1977-97 (fig. 6). Concentrations were at their highest in 1979 (1.62 ± 0.4 μg/g) and declined to their lowest concentrations in the mid-1990's (ie, 0.20 ± 0.14 μg in 1991). Concentrations of copper in surface sediment also decreased by half over the study period, from 86 ± 28μg/g in 1979 to 43 ± 10 μg/g in 1993 (fig. 6). In contrast, zinc concentrations in bed sediment showed no distinct trends over time, with a 20 year annual average of 147 ± 19 μg/g (fig. 6). Selenium and mercury concentrations were not significantly different between 1977-87 and 1992-97 (table 1).

Metal Trends in Bivalves

Mean annual concentrations of both silver and copper in *M. balthica* showed a strong trend of declining concentrations from the 1970's through 1991, despite high, distinctly seasonal, intra-annual variability (see also Cain and Luoma, 1990) (fig. 6). The highest annual mean concentrations of silver in *M. balthica* during the study was 103 ± 28 μg/g in 1978; silver concentrations were 3.6 ± 2.9 μg/g in 1997. The highest and lowest copper concentrations in *M. balthica* averaged 295 ± 115 μg/g in 1979 and 24 ± 13 μg/g in 1991, respectively. The annual mean concentrations of silver appeared to respond rapidly to the treatment upgrades that occurred at the PARWQCP. For example, mean concentrations of silver declined >50 percent between 1980 (109 ± 41 μg/g) and 1982 (45 ± 22 μg/g) after the first phase of plant improvements. They again declined >50 percent between 1987 (55 ± 30 μg/g) and 1989 (11 ± 7 μg/g), after the second phase. Copper concentrations show a more gradual decline over time (fig. 6).

Fluctuations in annual mean zinc concentrations in *M. balthica* between 1977 and 1997 were distinctly different from those observed with silver or copper (fig. 6). No unidirection trend was observed over the study period. Concentrations of zinc in *M. balthica* were relatively low in the mid-to late 1970's (277 ± 31 μg/g), annual average concentrations increased in the early 1980's (382 ± 74 μg/g), then declined again in 1987-1991 (212 ± 57 μg/g). The annual mean zinc concentration in 1988 was 179 ± 82 μg/g; in 1991 it was 179 ± 43 μg/g (fig. 6). Annual mean zinc concentrations increased again to 434 ± 94 μg/g in 1996. Thus, over the entire study period a nearly bimodal distribution of temporal zinc trends was evident.

While there appears to be some variation among years in the bioaccumulation of cadmium, chromium, nickel and vanadium, there is no significant trend in concentrations (fig. 5). The annual mean range from 1990-97 varied slightly for each metal: cadmium, 0.2-0.4 μg/g; chromium, 2-4 μg/g; nickel, 4-8 μg/g; vanadium, 1-4 μg/g. These metals were not analyzed prior to 1990.

The relationship between bioaccumulation and sediment concentrations were not sufficiently strong to be predictive (fig. 7), but the general trends in both indicators were similar. In general, the bivalves responded more strongly to changes in contamination than did sediments. Concentrations of silver and copper in sediments correlated significantly with concentrations in bivalves ($p<0.01$: $R^2=0.71$ and $R^2=0.50$, respectively). However, concentrations of zinc in sediments were not significantly correlated with concentrations in *M. balthica* among all data from the 20 year study (fig. 7).

Although concentrations of copper and silver in *M. balthica* declined substantially from the very high concentrations found in the 1970's, concentrations in the 1990's were not as low as the regional background for this element in this species. Figure 8 compares annual mean copper at the Palo Alto site in 1989 and in 1996 to concentrations in populations from two stations which are not directly affected by local copper discharges. Regional surveys conducted throughout the Bay show that the concentrations of copper at the two reference stations are among the lowest observed in San Francisco Bay (Luoma and Phillips, 1988). While these two sites probably contains

a regional signal of metal inputs to the Bay, they do not reflect the strong localized point source that occurs at the Palo Alto mudflat. The mean regional baseline for copper between these stations is 25 μg/g. These values compare to a mean copper concentration of 36.8 ± 12 μg/g observed at the Palo Alto site from 1988 - 1991 and a mean of 54.5 ± 23.3 μg/g observed for 1992 - 1997. Similarly determined regional baseline concentrations of silver and zinc were 0.5 μg/g and 200 μg/g, respectively.

The decline in concentrations of copper and silver in *M. balthica* between 1977 and 1990 was linked to declining metal concentrations and loads from the PARWQCP. Historic determinations of silver in effluents are probably unreliable. However, the more reliable determinations of annual mean concentrations of copper in PARWQCP effluent, and annual copper loads from the plant, were strongly correlated with annual mean copper concentrations in *M. balthica* for the 1977 - 1990 period (R^2 = 0.95; Fig. 9). Loads of copper from the PARWQCP continued to decline steadily between 1991-97 (fig. 3), but copper concentrations in *M. balthica* increased over this period. No significant correlation was observed between effluent copper and copper bioaccumulation in *M. balthica* for this period. Concentrations of silver and copper in *M. balthica* were 3.3 ± 3.4 μg/g and 24 ± 13 μg/g, respectively in 1991, but averaged 6.3 ± 0.96 μg/g and 59.8 ± 9.6 μg/g, respectively during the 1992-97 period. Loads of copper in effluents declined during this period (Fig. 3), from approximately 500 kg/yr to 187 kg/yr. Bioaccumulation of zinc did not correlate with zinc loads from the PARWQCP because there was no significant change in Zinc discharge over time (fig. 3).

In addition to local inputs of metals from the PARWQCP, additional inputs from outside sources must also be considered. Discharges from the San Jose and Sunnyvale Water Quality Control Plants are located within 10 km of the Palo Alto study site. *M. balthica* and surface sediments have been collected near-monthly since January 1994 from a shallow water site near the mouth of Coyote Creek, between the two discharges. Trends in metal bioaccumulation over this short period have generally been similar at the San Jose site and the Palo Alto site (Luoma and others, 1998). One exception has been episodic high concentrations of Hg found in sediments (and less so in clams) at

the San Jose site, presumably originating from pulse inputs into Guadalupe Slough or Coyote Creek. High mercury concentrations have not been observed at the Palo Alto site during these episodes. Thus, at least these localized inputs from nearby sources, do not influence metal concentrations at the Palo Alto station. The similarities in other metal concentrations that occur between the two sites points toward regional scale processes (such as inputs from urban runoff) as an important influence on sedimentary and bioavailable metal concentrations in South Bay in the 1990's (Luoma and others, 1998).

Because no interpretable, direct studies of inputs from urban runoff exist, both stream flow and precipitation were used as surrogate measures for metal loads entering the Bay from urban runoff. Annual mean concentrations of copper in *M. balthica* were significantly correlated with precipitation from 1989 - 1997 ($R^2 = 0.79$; fig. 10). The correlation with local stream flow was also significant, but less strong ($R^2=0.65$). However, uncertainties are introduced into the stream flow data by impoundments in the watershed of most streams. Concentrations of copper in *M. balthica* did not correlate significantly with precipitation prior to 1989, when correlations with inputs from the PARWQCP suggest the treatment plant was the predominant source of metal to the mudflat (fig. 9). The long-term zinc concentrations in *M. balthica* correlated significantly with precipitation ($R^2 = 0.53$; p<0.001) and with stream runoff ($R^2 = 0.58$; p,0.001) (fig. 11a, b). However, the relations were not highly predictive. Visual inspection of the trend indicated that the correlations were partly confounded by high zinc concentrations retained in the animals for a year or more, after each wet year (for example, 1984-85; 1987; 1994).

Trends in Reproduction

Nichols and Thompson (1985) first demonstrated the cycle of reproductive activity typical of *M. balthica* from San Francisco Bay mudflats from data collected in 1983-84. Reproductively active individuals occurred throughout the year at all mudflats; the annual average percentage of reproductively active individuals was 40-60 percent (fig.

12). The proportion of individuals reaching maturity increased during the early part of each year (January - April) with a second phase of increase possible during the fall. An anomalous pattern of reproductive activity was observed at the Palo Alto site in 1983-84 by Nichols and Thompson (1985), in that the fall increase in reproductive maturity was absent. A typical reproductive cycle for *M. balthica* was observed at the Palo Alto site in 1988-89 in the animals analyzed for the present study. Nineteen of 24 consecutive months were sampled during this period, and during most of those months, >60 percent of the individuals collected were reproductively active (fig. 12). Maximum reproductive maturity (70 - 100 percent of individuals) was observed in the early part of each year and in the fall of each year.

During the period when silver and copper concentrations were at their highest, the proportion of reproductively mature individuals was very low. Among 15 of 18 consecutive months sampled in 1974-75, reproductively mature individuals were found in only three months, and the proportion of animals showing mature reproductive tissues never exceeded 50 percent (fig. 12). On average, less than 10 percent of the animals were reproductive during this period (fig. 13). During the 1979-81 period, reproductively active individuals occurred in 9 of the 20 months sampled (fig. 12), but the average occurrence of reproductive maturity over the study period was only 17 percent (fig. 13). It appeared that *M. balthica* was not reproducing successfully at Palo Alto between 1974 and 1981. However, as metal concentrations began to decrease in the mid- to late 1980's, the proportion of individuals that reached reproductive maturity also increased (fig. 12). In the 28 months sampled during the 1983-85 period, only 4 of the months contained samples were 100 percent of the individuals collected showed no reproductive activity (fig. 12). On average, >50 percent of *M. balthica* collected during this time period was reproductively active (fig. 13). The decline in metal loads a significant environmental variable that corresponds to this change, although exceptionally high rainfall and large inflows that during 1983 may have also contributed (perhaps greater allochthonous food inputs and/or slightly diluted metal concentrations).

DISCUSSION

The area of influence near the PARWQCP was characterized by contaminated sediments and severe contamination of resident biota with silver and copper in the late 1970's and early 1980's (Thomson and others, 1984). Contamination with silver and copper also was documented in resident biota throughout South Bay at that time (Luoma and Cain, 1979; Luoma and Phillips, 1988), but to a lesser extent than at the Palo Alto site (that is, the extreme local contamination was accompanied by more moderate regional contamination). In addition, copper and silver contamination was reported in bivalves transplanted into South Bay (Martin and others, 1984; Smith and others, 1986) in the early 1980's.

With improvements in the waste treatment processes at the PARWQCP, silver and copper contamination in resident biota declined at the study site, near the plant outfall. Public investment in advanced waste treatment at the PARWQCP clearly resulted in reduced sediment contamination and reduced exposure of local, resident benthos to these contaminants. The rapid response to the reduction in contaminant discharge near the PARWQCP was probably enhanced by substantial seasonal mixing and renewal of sediments (Thomson-Becker and Luoma, 1985), typical of shoal environments in South Bay (Fuller, 1982). Recent studies of sediment cores from North and Central San Francisco Bay suggest that trends of reduced contamination are beginning to appear on a regional scale for some metal pollutants, but not for others (Hornberger and others, in press). The strong decline in copper concentrations observed locally at Palo Alto was not evident in these cores, although some decline in silver concentrations was observed. Sediment cores have not been studied in South Bay partly because areas of continual sediment deposition are rare (Fuller, 1982). Dissolved copper and silver concentrations remain higher in South Bay than found elsewhere in San Francisco Bay, however (Flegal and others, 1991; Smith and Flegal, 1993). In 1990, dissolved silver concentrations were equivalent to those found in the highly contaminated San Diego Bay (Flegal and Sanudo-Wilhelmy, 1993). Stephenson and Leonard (1994) recently reported a significant trend of declining silver concentrations

(but not copper) in transplanted mussels *(Mytilus californianus)* at a California mussel watch station in South San Francisco Bay.

Three decades after passage of the Clean Water Act, improvements in contamination on a national scale are following trends with similarities to those reported at Palo Alto. Throughout the country there are examples where severe contamination has been reduced, but a significant contaminant residual remains that has been difficult to eliminate (Hanson, 1998). Sediment cores in lakes and estuaries show declines in lead concentrations since maxima in the 1960's and 1970's, but recent trends are ambiguous (Callendar and Van Metre, 1997; Hornberger and others, in press). PCB concentrations in biota of the Great Lakes declined 40 -80 percent in the first 20 years following regulation, but rates of decline have apparently slowed (Smith, 1995; Stow, 1995; Hebert and others, 1997). While the biological impacts of the earlier, severe PCB contamination were unambiguous (in retrospect) in the Great Lakes, the biological significance and the solutions for the remaining contamination remain controversial (Cooper, 1995; Eder and Schmidt, 1995; Kitchell, 1995).

As in the case of the Great Lakes, management practices in San Francisco Bay appear to have succeeded in eliminating some of the most obvious contamination from point sources since 1970 (Monroe and Kelly, 1992). However, since 1990 reductions of silver and copper contamination have not occurred near the PARWQCP. Inputs from historical contamination in sediments may contribute to the continuing biologically available contamination in South Bay (Smith and Flegal, 1993) and in other ecosystems (Smith, 1995). Complicated shifts in the relative importance of sources of contamination also can affect trends in bioaccumulated contaminants (Hebert and others, 1997; Gobas and others, 1995). At the Palo Alto site, the most important source of the contamination appears to have been controlled, but what was once, perhaps, a secondary source now appears to be dominant. Correlative evidence suggests that the point source discharges of the PARWQCP controlled annual mean copper concentrations through approximately 1990. Assuming that precipitation is a surrogate for urban runoff loadings (or, less likely, for atmospheric inputs), non-point source inputs appear to have controlled copper concentrations in *M. balthica* from 1990 to present. In contrast, bioaccumulation of zinc

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at Palo Alto has correlated with precipitation and stream inflows since 1977. This suggests that urban runoff was the source controlling year-to-year variations in zinc bioavailability in South Bay throughout the study period. Understanding the sources of the remaining contamination in South Bay is critical to future management of point sources and non-point sources. The biomonitoring data from Palo Alto provide unique evidence that rainfall-associated sources release biologically available copper and zinc to South Bay and that inputs associated with runoff have influenced concentrations in benthos more significantly than point source inputs, since about 1990. A better understanding of the rainfall-associated inputs of copper and zinc is clearly the next step in this ecosystem.

A number of adverse biological effects have been observed in resident populations of animals at the Palo Alto mudflat since the late 1970's. During our on-going collections, the population of *M. balthica* periodically disappeared or became very depauperate in the late 1970's and early 1980's when metal concentrations were at their highest (based on anecdotal "clams per unit effort" data from our collections). Population densities of *M. balthica* did not appear to fluctuate too greatly at adjacent South Bay mudflats. Studies conducted during this time period determined that the population of *M. balthica* at the Palo Alto site was tolerant to copper (and silver; unpublished data) compared to populations from less contaminated mudflats (Cain and Luoma, 1985). In 1980, a metal-binding protein having the characteristics of metallothionein was identified in *M. balthica* at the Palo Alto site (Johansson and others, 1986). Metallothionein (MT) appears to be important in the detoxification of metals, including silver and copper (Mason and Jenkins, 1995). At Palo Alto, Johansson and others, (1980) observed an apparent metal saturation of the MT pool in *M. balthica* at high tissue concentrations, and a spillover of silver and copper into the lower molecular weight protein pool. Spillover of MT bound metal to other proteins has been associated with adverse biological affects, including reproductive potential (Jenkins and Mason, 1988). Animals that have the ability to synthesize MT and to sequester excess concentrations of metals may express metal tolerance (Mulvey and Diamond, 1991; Klerks and Levinton, 1989). These studies not only support the effects of metal-specific stress on the Palo Alto

mudflat, but provide an explanation why the tolerant population survived in the presence of large scale population-level stress.

The present study shows that *M. balthica* populations were probably not reproducing successfully between 1974 and 1981, and possibly, reproduction failed periodically through 1988. The most likely cause of reproduction impairment was exposure to copper and silver (based upon the combination of exposure, biomarker and population level evidence). Immigration of pelagic larvae from reproducing populations elsewhere, and selection of individuals tolerant to copper and silver from the assemblage of immigrating recruits is the most likely reason that *M. balthica* populations persisted at this mudflat in the 1970's and early 1980's (Luoma and Cloern, 1982). Although sediment characteristics, concentrations of other metals, and other potential stressors (salinity, food availability as indicated by carbon concentrations in sediments) varied over the period of the study, only the trend in metal contamination co-varied with the stress in early years and recovery of reproductive capabilities in later years.

Additional lines of evidence suggest that elevated metal concentrations were the primary stressor to South San Francisco Bay and were not limited to the Palo Alto mudflat. A study conducted by Stephenson (1987) transplanted juvenile oysters to South San Francisco Bay to study the effects of Tributyl tin (TBT). Instead of shell deformations, they found greatly reduced growth in the transplanted animals, an expected effect from metals like copper or silver rather than TBT. Martin and others (1984) found greatly reduced "scope-for-growth" in mussels (*Mytilus edulis*) transplanted toward extreme South Bay, correlating with, among other variables, elevated silver concentrations in mussel tissues.

Contamination of sediments at the Palo Alto site with copper and silver never exceeded guidelines for sediment quality criteria between 1977 and 1997. Long and others (1995) reported an 84 percent incidence of adverse biological effects in studies where copper concentrations in sediments exceeded 270 μg/g (the "ERM" guideline) and a 29 percent incidence of effects when copper concentrations were between 34 and 270 μg/g (>"ERL" guideline). At their maximum, annual mean copper concentrations in sediments at the Palo Alto site were 86 ± 28 μg/g. Long and others (1995) reported a

93 percent incidence of adverse effects when sediment silver concentrations exceeded 3.7 μg/g (ERM) and a 32 percent incidence of effects when silver concentrations in sediments were between 1.0 μg/g and 3.7 μg/g (>ERL). Maximum mean silver concentrations in sediments at Palo Alto were 1.62 $\pm$ 0.38 μg/g (HCl extraction). The maximum sediment concentrations of silver and copper at the Palo Alto site coincided with an etiology in *M. balthica* strongly indicating sublethal contaminant disturbance and population stress. The adverse biological effects at the Palo Alto site occurred in the window between NOAA's ERL and ERM guidelines, in the cases of both copper and silver. Such results suggest that, if the NOAA sediment guidelines are to be employed, the ERL-ERM window may be the guideline more indicative of the complicated manifestation of metal stress in natural systems. Tissue residues also may provide an indicator of stress thresholds. While the amount of reproductive data for *M. balthica* is relatively small, we can estimate that reproduction was most successful after silver concentrations in clam tissues fell below 20 μg/g and copper concentrations fell below 95 μg/g. Successful reproduction in 1983 coincided with silver tissue concentrations of 56 μg/g and copper tissue concentrations of 195 μg/g.

Recent reviews have questioned the value of bioaccumulation as a tool in evaluating or monitoring contamination (Chapman and others, 1996). Bioaccumulation in a resident species, alone, cannot provide a full explanation of the ecological effects of contamination. However, bioaccumulation in a resident species fits the most important criteria for long-term ecological studies (Franklin, 1989; Likens, 1992). Determinations of bioaccumulation are practical over a long period of time, are relatively inexpensive, and the data are relatively unambiguous to interpret. In this study we have shown that contaminant bioaccumulation provides direct feedback about the success or failure of changes in mitigation of pollutant inputs such as changes in wastewater treatment. Such data, when collected in conjunction with relevant environmental information (discharge data; primary hydrologic data; basic geochemical data), can provide reasonable hypotheses about the relative importance of different sources of contamination. Most important, as an indicator of exposure or dose, bioaccumulation data from a resident bioindicator can provide the basis for evaluating at least

organismic-level and population-level effects of a contaminant.

ACKNOWLEDGMENTS

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Table 1. Average metal concentrations for surface sediment during four time periods. Concentrations are in micrograms per gram dry weight. Iron measured as percent dry weight.

Time Period	Ag	Cu	Fe	Hg	Mn	Se	Zn	%TOC
1977-81	1.4 ± 0.4	66.7 ± 21.1	4.6 ± 1.3	0.39 ± 0.07	1020 ± 400	0.26 ± 0.11	142 ± 45	1.2 ± 0.4
1982-87	0.7 ± 0.2	51.5 ± 11.2	3.6 ± 0.8	0.36 ± 0.02	990 ± 410	0.5 ± 0	141 ± 64	1.3 ± 0.3
1988-91	0.6 ± 0.1	52.3 ± 6.6	4.6 ± 0.6	—	1180 ± 330	—	128 ± 26	1.3 ± 0.2
1992-97	0.38 ± 0.17	45.1 ± 8.7	4.8 ± 1.4	0.31 ± 0.06	1089 ± 335	0.41 ± 0.12	137 ± 19	1.3 ± 0.3
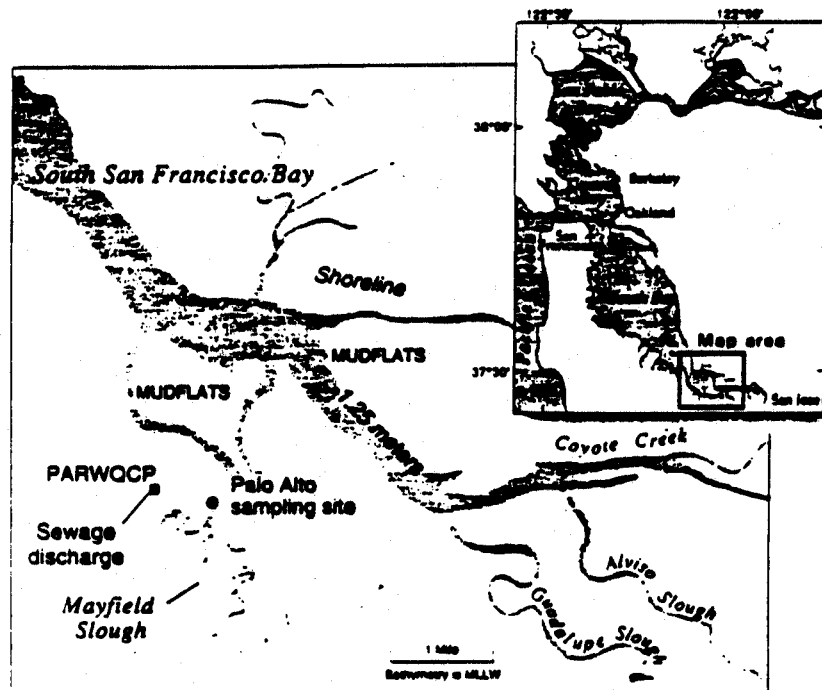
1. The Palo Alto sampling site in South San Francisco Bay, California.
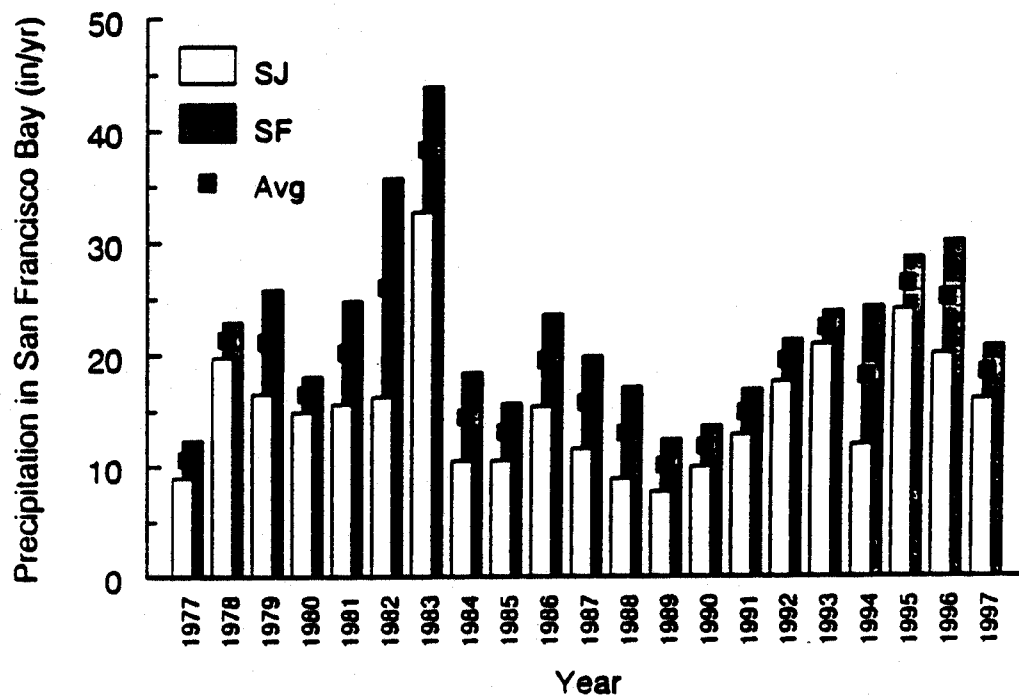
2. Annual total precipitation in San Francisco (dark bars) and San Jose (light bars) from 1977-97. Estimated annual total precipitation for the South Bay (closed squares) is calculated using the average of San Francisco and San Jose precipitation measurements.
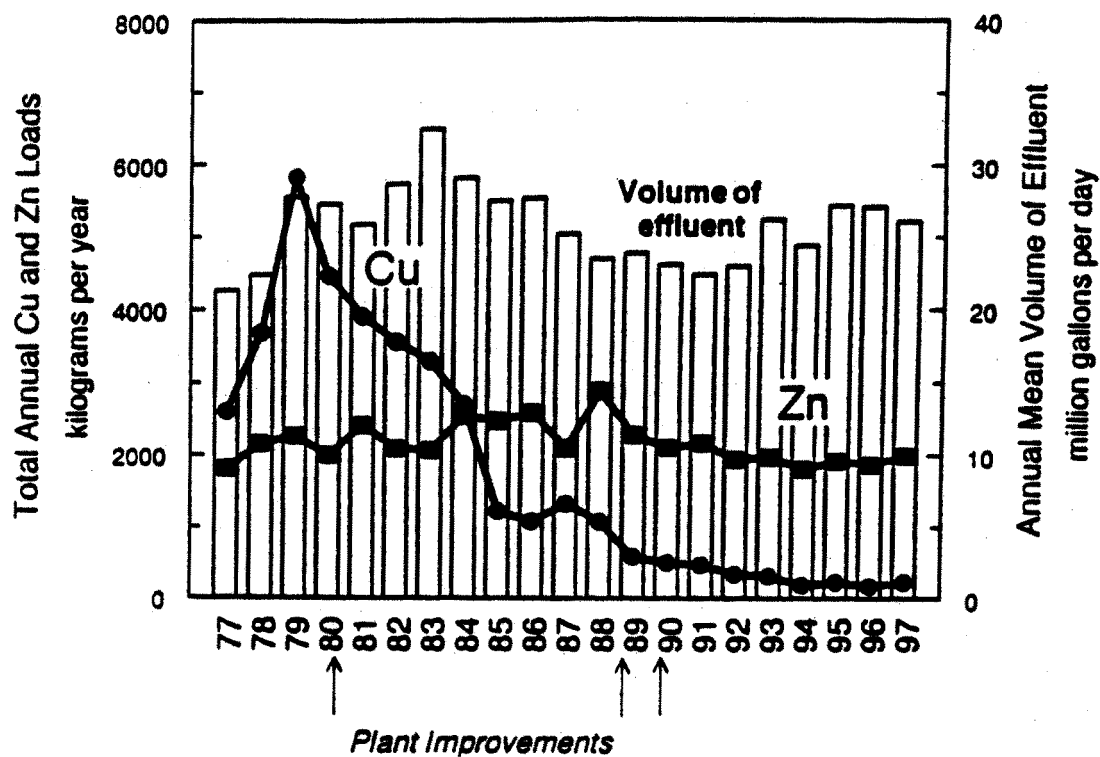
3. Annual mean volume of effluent from the Palo Alto Regional Water Quality Control Plant (PARWQCP) vs. total annual copper and zinc loads. Arrows at the bottom of the graph indicate years when treatment improvement were put into place. In 1980, trickle filters and nitrification processes were added. Increased retention time and clarifiers were added in 1988. In 1989, source control of silver was implemented.

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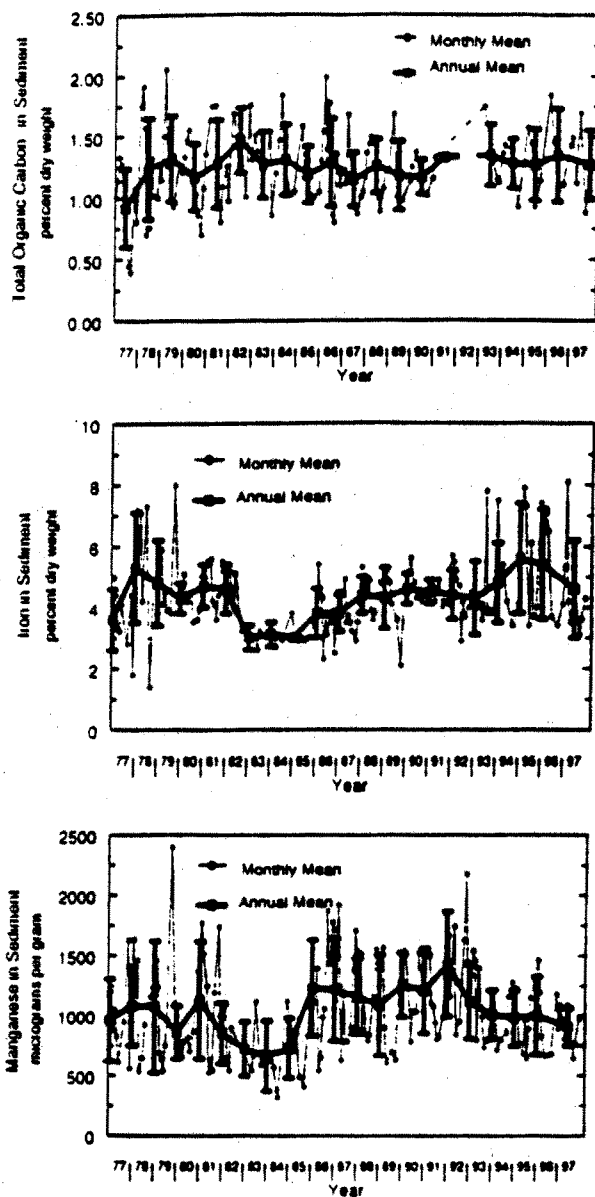
4. Annual trends of Total Organic Carbon (% TOC), iron concentrations (by % dry weight) and manganese concentrations (μg/g dry weight) in surface sediments from the Palo Alto mudflat from 1977-97. Monthly mean values (closed circles) are compared to yearly averages (closed squares).

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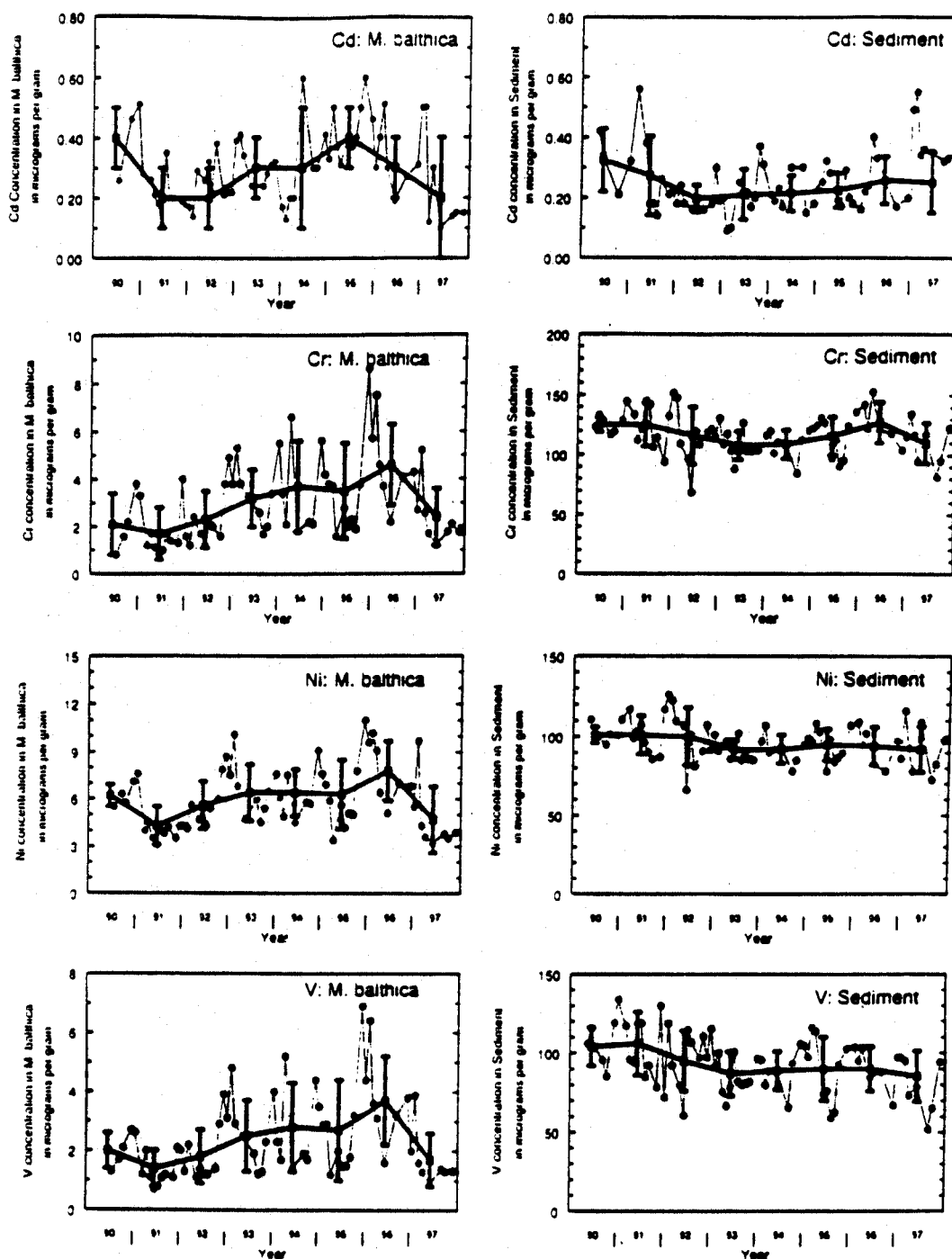
5. Annual trends of cadmium. chromium, nickel, and vanadium in *M. balthica* and surface sediments from the Palo Alto mudflat from 1990-97. All concentrations are reported in μg/g dry weight. Monthly mean values (closed circles) are compared to yearly averages (closed squares).
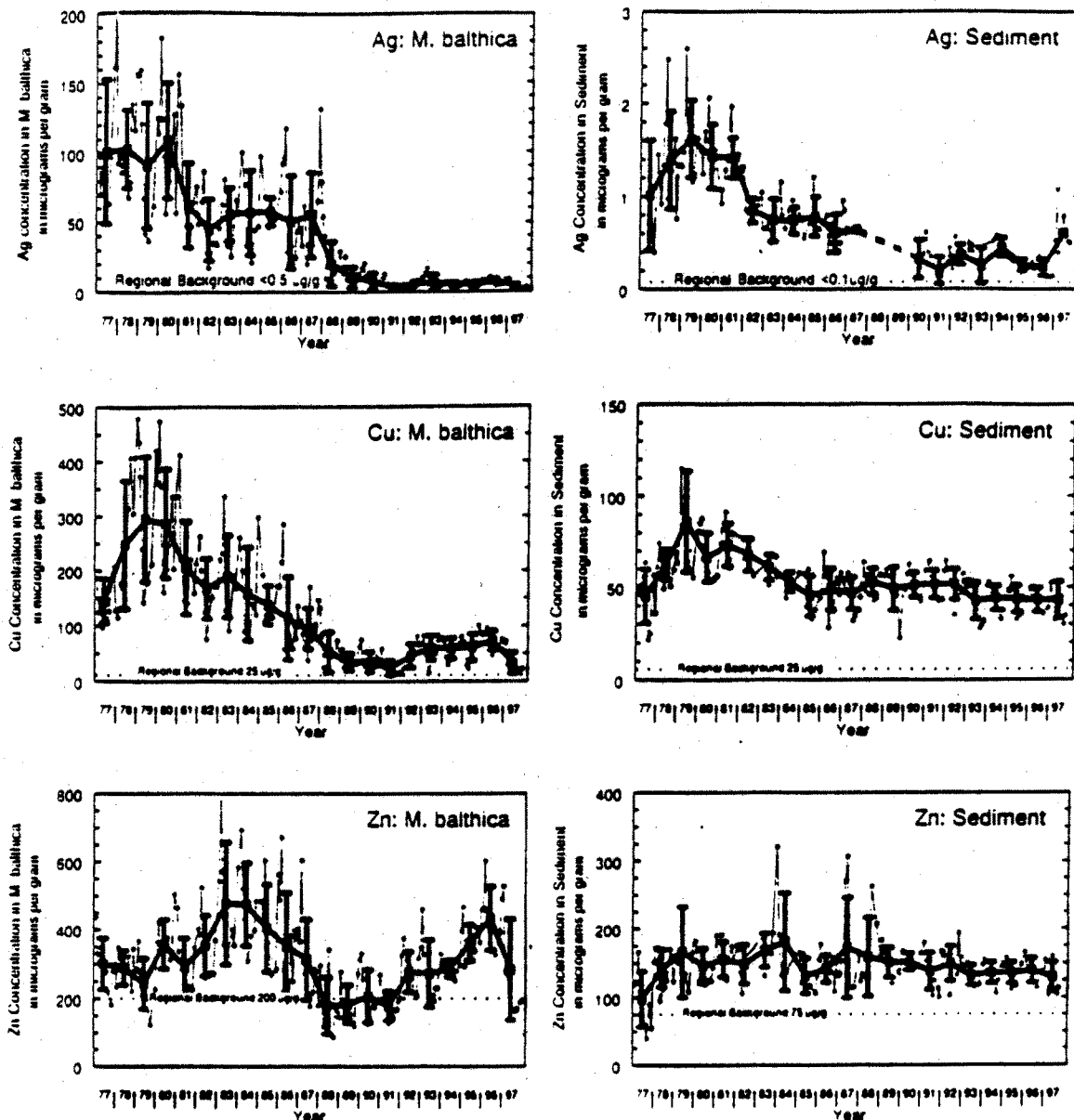
6. Annual trends of silver, copper, and zinc in *M. balthica* and surface sediments from the Palo Alto mudflat from 1977-97. All concentrations are reported in µg/g dry weight. Monthly mean values (closed circles) are compared to yearly averages (closed squares). Regional background values are indicated for each element by the dashed line.

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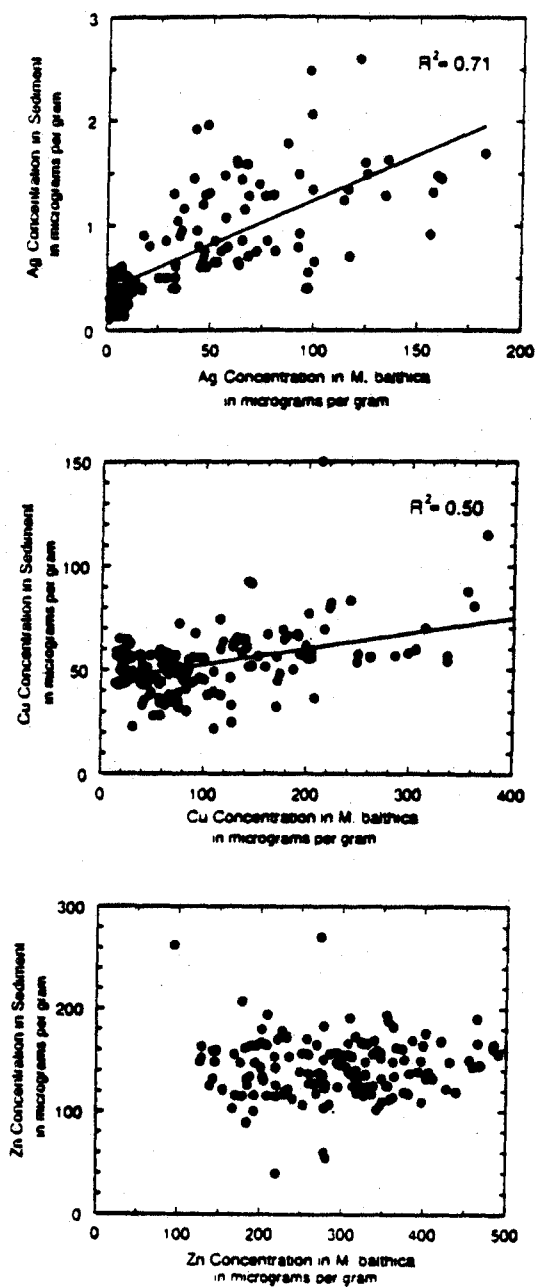
7. Correlations between surface sediment concentrations and bioaccumulation of silver, copper and zinc by *M. balthica*. All concentrations are reported in µg/g dry weight. Data includes all monthly values from 1977-97.
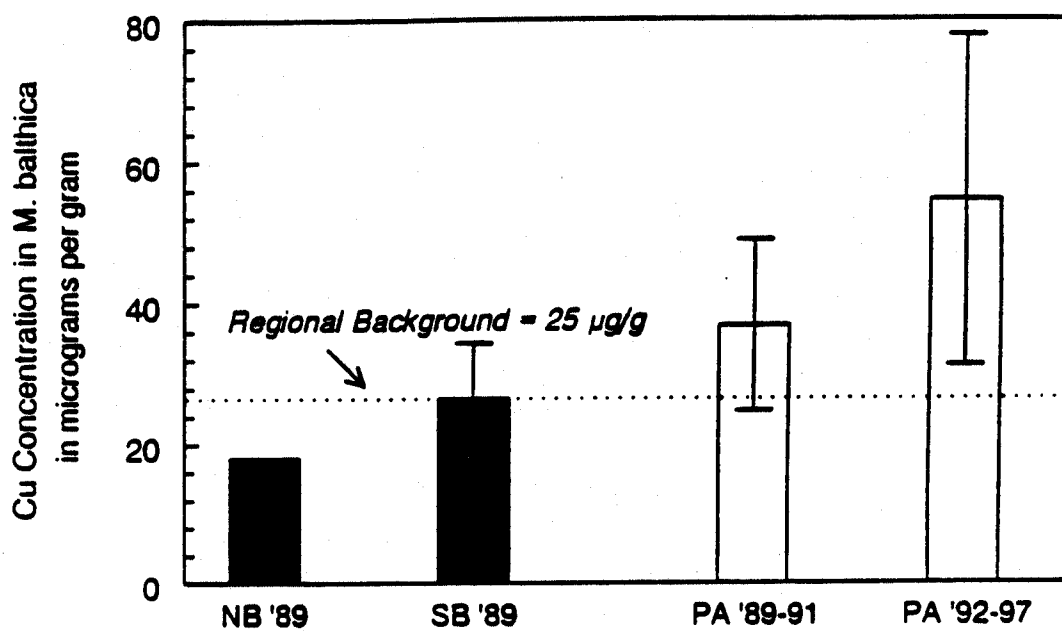
8. Comparison of copper concentrations in *M. balthica* at the Palo Alto site (PA, 1989-91 and PA, 1992-96) to reference sites in North San Francisco Bay (NB, 1989) and South San Francisco Bay (SB, 1989).

9. Correlation between total annual copper load from the PARWQCP and annual average copper in *M. balthica*. Closed circles indicate the positive correlation ($R^2 = 0.95$; $p < 0.001$) from 1977-90. Values from 1991-97 (open squares) show no relationship with copper loads from the PARWQCP.
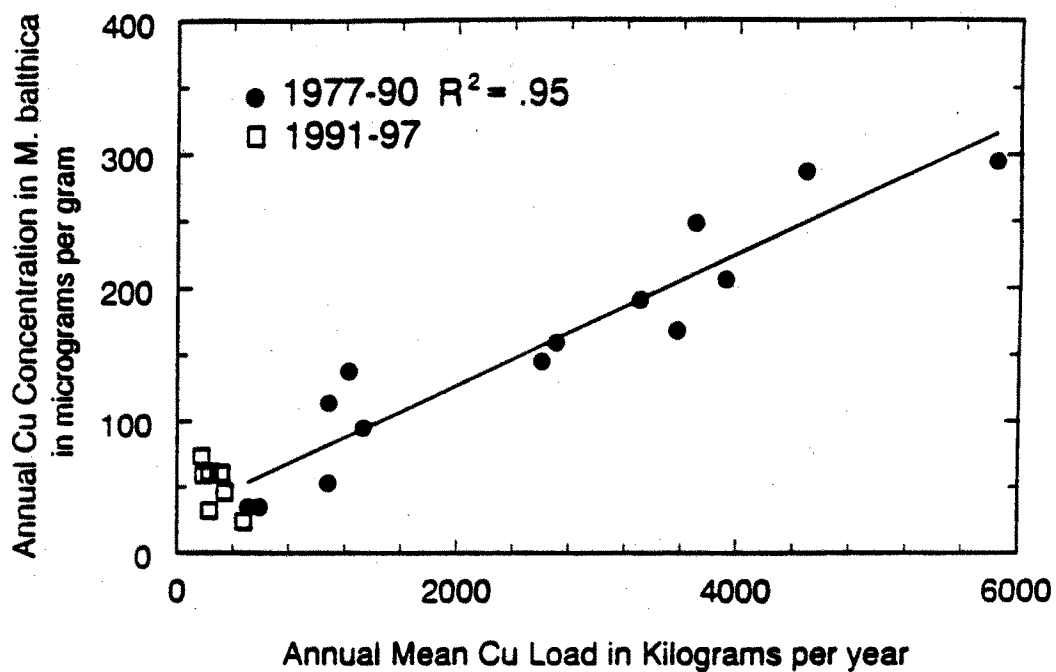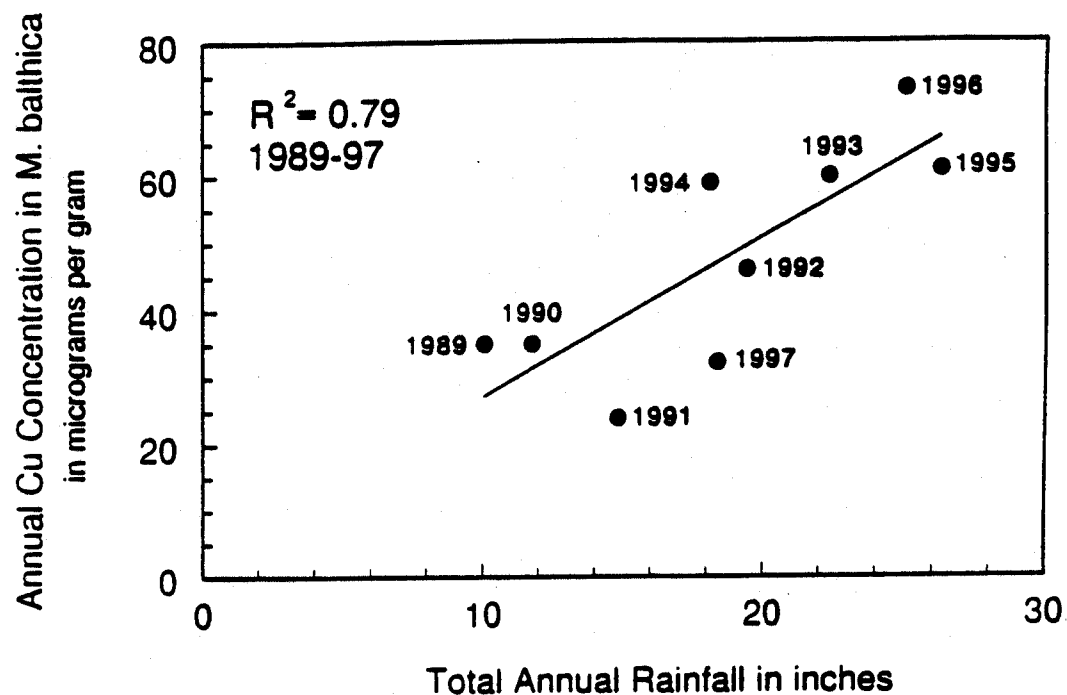
10. Correlation between annual average copper concentration in *M. balthica* and total annual rainfall. Precipitation is based on the average of San Francisco and San Jose (see methods). Closed circles indicate the positive correlation ($R^2 = 0.79$; $p<0.001$) from 1989-97.

(a)

(b)
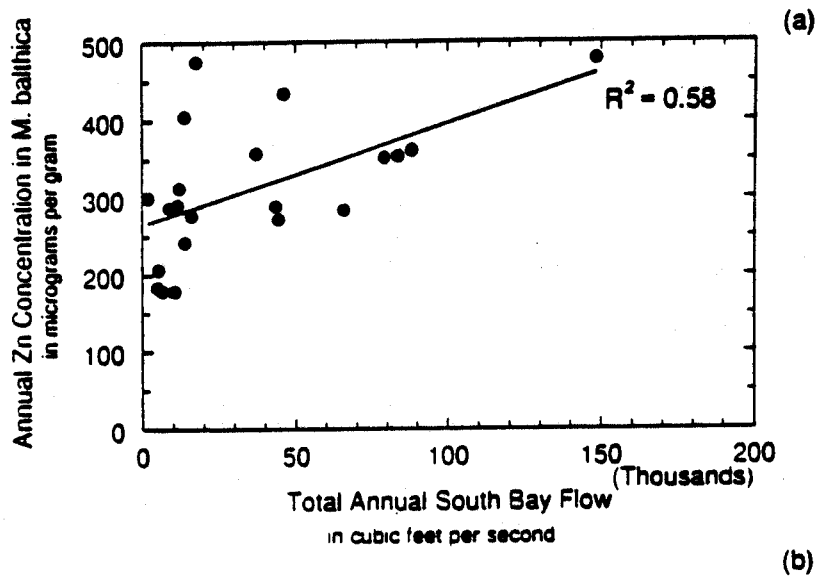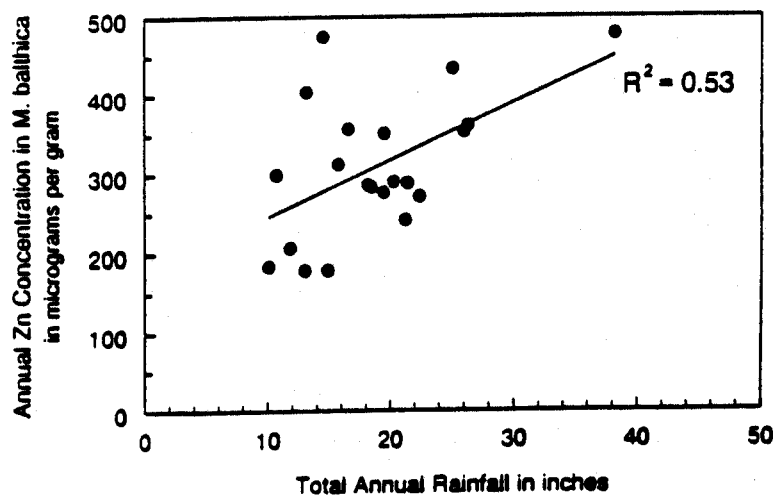
11a. Annual mean concentrations of zinc in *M. balthica* correlated against total annual rainfall (estimated as an average between San Francisco and San Jose). Closed circles indicate the positive correlation (R^2= 0.53; $p<0.001$) from 1977-97. 11b. Annual mean concentrations of zinc in *M. balthica* correlated against total annual South Bay flow (see methods). Closed circles indicate the positive correlation (R^2= 0.59; $p<0.001$) from 1977-97.
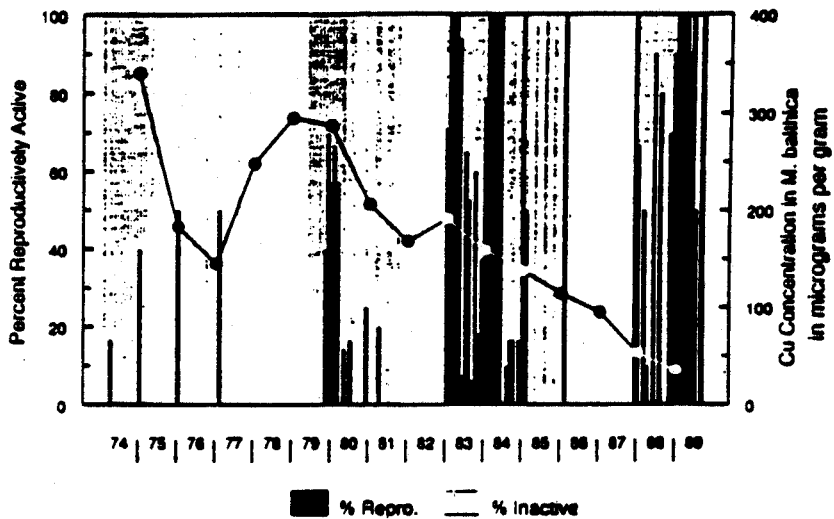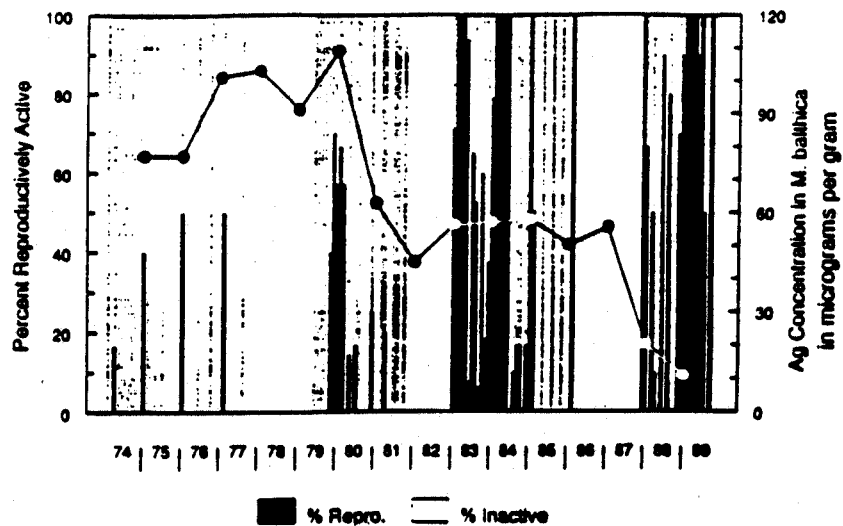
12. Stacked bar graph showing the percent of *M. balthica* that were reproductive (left axis) during four periods (1974-76; 1979-81; 1983-85; 1988-89). Gray portion of the bar represents the proportion of clams that were inactive. Dark areas of the bar represents percent of clams reproductive (See methods for description of each category). Annual mean concentrations of silver and copper (plotted against the right axis) are shown as a closed circle.
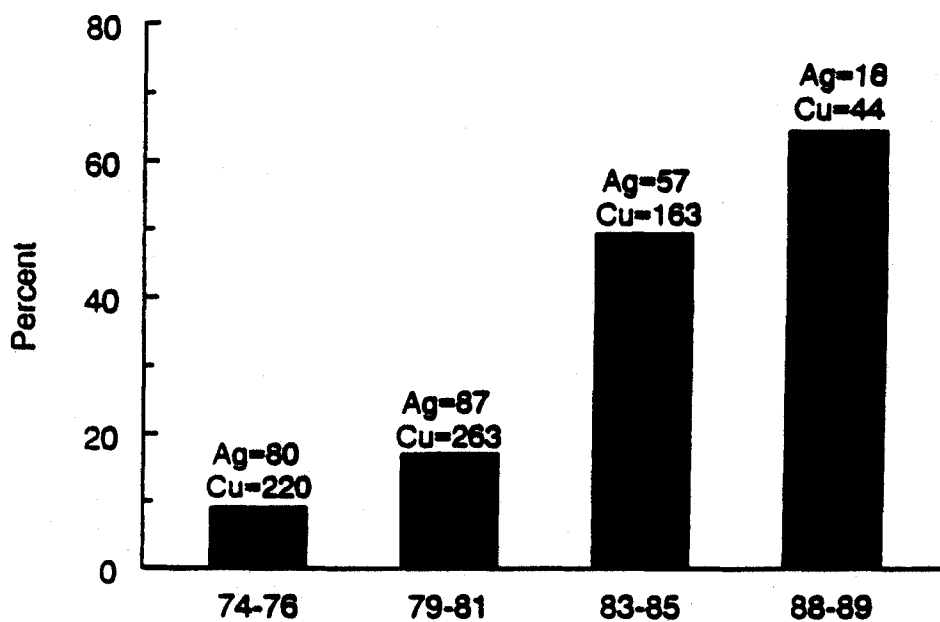
13. The percent of *M. balthica* that were reproductively active during four time periods at the Palo Alto study site. Average concentrations of silver and copper are in units of micrograms per gram dry weight.

Appendix C
Toxicity Profiles for
Copper and Nickel

APPENDIX C
TOXICITY PROFILES

1.0   INTRODUCTION

This impairment assessment establishes copper and nickel concentrations that pose an unacceptable potential for adverse ecological effects to identified receptors of concern. This appendix identifies and provides rationale for selecting toxicity benchmarks for copper and nickel that may be used to derive avian and mammalian reference toxicity values (RTVs).

1.1   APPROACH

For this impairment assessment, the RTV is defined as the dose of a chemical (i.e., copper, nickel) that is protective of a particular wildlife receptor. To provide a conservative assessment, the RTV is the dose at which no chronic effect is observed, and above which effects just begin to occur. The toxicity benchmark is defined as the dose that is administered to the test species and is used to derive a wildlife-specific RTV:

$$\text{RTV}_{\text{wildlife}} = \text{Toxicity Benchmark}_{\text{test spp}} \bullet \text{UF}$$

Uncertainty factors (UFs) may be applied to complete the extrapolation (e.g., species-to-species, endpoint-to-NOAEL). For example, if an acute reproductive LOAEL of 1-milligram of copper per kilograms-day (1 mg[Cu]/kg-day) for the laboratory rat was selected as the toxicity benchmark, then the following expression may be used to derive a chronic NOAEL-equivalent RTV for the harvest mouse:

$$\text{RTV}_{\text{Harvest Mouse}} = \text{Toxicity Benchmark}_{\text{Rat}} \bullet \text{UFs}$$

$$= 1 \text{ mg[Cu]/kg-day}_{\text{Rat}} \bullet \text{UF}_{\text{Rat-to-Harvest Mouse}} \bullet \text{UF}_{\text{LOAEL-to-NOAEL}} \bullet \text{UF}_{\text{Acute-to-Chronic}}$$

This approach is consistent with available regulatory risk assessment guidance (DTSC 1996; U.S. EPA 1997).

1.2   PREFERRED TOXICITY DATA

Only toxicity studies that reported all of the following data were used to develop toxicity benchmarks:

- Chemical administered;
- Test organism;
- Administered dose(s);
- Exposure duration;
- Exposure route;
- Effect or response;
- Sample size; and
- Full citation or full citation of source.

Reproductive impairment, developmental abnormalities, and mortality were the preferred toxicological responses because they can be directly related to reproductive success (i.e.,-the ability of individuals to

leave viable offspring to the next generation) and the persistence of wildlife populations. Use of reproductive and developmental toxicity data is recommended by guidance (DTSC 1996; U.S. ACE 1996). Whenever possible, chronic NOAEL values for either reproductive impairment or developmental abnormality were used to develop RTVs for this assessment.

Based on a review of compiled toxicity data, doses that resulted in mortality were often greater than doses that resulted in reproductive or developmental effects. Therefore, only when reproductive or developmental data were not available were chronic mortality data considered. Physiological (e.g., enzyme activity), systemic (e.g., organ weight), and behavioral responses were less preferred because it was often difficult to relate these responses to quantifiable decreases in reproductive success or the persistence of wildlife populations. Tumorigenic and carcinogenic toxicity studies were not considered ecologically relevant and were not used to develop toxicity benchmarks because debilitating cancers in wildlife are exceedingly rare under field conditions. However, physiological, systemic, behavioral, tumorigenic, and carcinogenic studies were used to support selection of toxicity benchmarks derived from reproductive and developmental studies.

Studies wherein copper or nickel was administered via an "unnatural" route of exposure (e.g., injection, implantation) were not considered because these routes cannot be directly related to exposures in the field. For the purposes of this assessment, only doses administered via ingestion were considered— ingestion is typically the predominant route of exposure in the field.

Ecologically relevant study features that were used to select among several germane reproductive or developmental toxicity studies include those in which:

- Wildlife species were examined in the study;

- Doses were administered during critical and sensitive periods (e.g., during gestation) and/or effects on sensitive life stage (e.g., effects on fetuses, embryos) were examined;

- Chronic exposures (> 50% of the life span) or doses were administered through most of the reproductive period;

- Use of a serial dosing regime, especially a serial dosing regime in which both a NOAEL and LOAEL were reported;

- Large "per treatment" sample sizes were examined; and

- A description and the results of statistical analyses were performed.

Not all effects observed in toxicity studies were considered to be "ecologically adverse" effects. To ensure consistency among toxicity benchmarks and with regulatory practices, 20 percent reductions or less in sublethal effects were not considered ecologically significant effects. In brief, most regulatory criteria are based on concentrations that cause effects that are statistically different from controls; these concentrations generally correspond to greater than 20 percent effects (Will and Suter 1995). In addition, generally differences in the field must be greater than 20 percent to be reliably detected by sampling or monitoring efforts that may be used to verify the assessment (Will and Suter 1995).

1.3 SOURCES OF TOXICITY DATA

Available toxicity data from numerous sources were reviewed (Table C-1). To facilitate queries, all relevant toxicity data were compiled into an electronic database.

Table C-1
Selected Sources of Toxicity Data

DATABASES

- Hazardous Substances Data Bank (HSDB). National Library of Medicine, National Toxicology Information Program. Bethesda, MD.

- Integrated Risk Information System (IRIS). U.S. EPA, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office.

- Registry of Toxic Effects of Chemical Substances (RTECS). National Institute for Occupational Safety and Health (NIOSH), Washington, D.C.

COMPILATIONS

- Agency for Toxic Substances and Disease Registry (ATSDR). 1997. *Toxicological Profiles*. On CD-ROM. CRC Press. U.S. Public Health Service. Atlanta, GA.

- Calow, P. (ed.). 1994. *Handbook of Ecotoxicology*. Volume 2. Blackwell Scientific Publications. London, England.

- Eisler, R. 1985-1993. *Hazards to fish, wildlife, and invertebrates: A synoptic review*. U.S. Fish Wildlife Service Biological Reports

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- Sample, B.E., D.M. Opresko, and G.W. Suter II. 1996. Toxicological benchmarks for wildlife: 1996 Revision. Prepared for the U.S. Department of Energy.

- Schafer, E.W. 1972. The acute oral toxicity of 369 pesticidal, pharmaceutical and other chemicals to wild birds. *Toxicol. Appl. Pharm*. 21: 315-330.

- U.S. Environmental Protection Agency (U.S. EPA). 1995. Great Lake Water Quality Criteria Documents for the Protection of Wildlife. EPA 820/b-85/008. Office of Water. Washington, D.C.

- U.S. Navy (U.S. Navy). 1997. Development of toxicity reference values as part of a regional approach for conducting ecological risk assessments at naval facilities in California. Draft Technical Memorandum. Prepared for the U.S. Navy

- Venugopal, B., and T.D. Luckey. 1978. *Metal Toxicity in Mammals. 2*. Plenum Press. New York, NY.

PRIMARY LITERATURE

- Over 400 citations

1.3.1 Mammals

Toxicity data for mammals (primarily rat and mouse) are relatively abundant because small mammals are often used to develop toxicity benchmarks for humans. References for reproductive impairment and developmental abnormality data came primarily from Sample *et al.* (1996), U.S. Navy (1997), U.S. Fish and Wildlife Service (USFWS) *Contaminant Hazard Reviews* (Eisler 1985-1993), Integrated Risk Information Service (IRIS) database, Registry of Toxic Effects of Chemical Substances (RTECS) database, *Sax's Dangerous Properties of Industrial Materials* (Lewis 1992), *Great Lakes Water Quality Initiative Criteria for the Protection of Wildlife* (U.S. EPA 1995), and Agency of Toxic Substances and *Disease Registry* (ATSDR) *Toxicological Profiles* (ATSDR 1997 on CD-ROM). Toxicity benchmarks were derived from the study selected among the most ecologically relevant studies.

1.3.2 Birds

Toxicity data for birds (primarily chicken, mallard, and quail) are limited primarily to metals and chlorinated pesticides. A large portion of these toxicity data are related to mortality (primarily acute mortality). References for reproductive impairment and developmental abnormality toxicity data for birds came primarily from Sample *et al.* (1996), U.S. Navy (1997), USFWS hazard reports (Eisler 1985-1993), *Great Lake Water Quality Initiative Criteria for the Protection of Wildlife* (U.S. EPA 1993b), and the Registry of Toxic Effects of Chemical Substances (RTECS) database. Toxicity benchmarks were derived from the most ecologically relevant studies.

1.4 EXTRAPOLATION FROM TOXICITY BENCHMARKS TO REFERENCE TOXICITY VALUES

Use of uncertainty factors may be employed to provide a conservative RTV. This is standard practice where there is limited toxicity data. Consequences related to the use of uncertainty factors are discussed in Section 1.5.

Whenever possible, use of wildlife species-specific toxicity data are recommended. However, applicable wildlife species-specific data are rarely available. Thus, when wildlife-specific toxicity data are not available, toxicity benchmarks for test species may be adjusted for representative wildlife species using the following allometric relationships for birds and mammals (Sample *et al*. 1996):

$$RTV_{Rep\ Spp} = Toxicity\ Benchmark_{Test\ Spp}\ x\ (BW_{Test\ Spp}\ /\ BW_{Rep\ Spp})^{1/4} \qquad \text{...for mammals}$$

$$RTV_{Rep\ Spp} = Toxicity\ Benchmark_{Test\ Spp}\ x\ (BW_{Test\ Spp}\ /\ BW_{Rep\ Spp})^{0} \qquad \text{...for birds}$$

The duration of exposure is critical in assessing the potential for adverse effects to wildlife. However, no clear guidance exists dividing subchronic and chronic exposures. Therefore, chronic exposures were defined as greater than 50 percent of the life span of mammalian wildlife representative species. Little information exists concerning the life span of birds used in toxicity studies and little standardization of study duration has been established for avian toxicity tests (Sample *et al.* 1996). Therefore for birds, exposures greater than 10 weeks were considered chronic; exposures less than 10 weeks were considered subchronic. These definitions are more conservative than the definition provided in technical support provided by U.S. EPA Region 9's Biological Technical Advisory Group (BTAG) (chronic exposures defined as greater than 10% of the life span) and are consistent with the U.S. Department of the Interior (DOI) (Sample *et al.* 1996).

In addition to duration, the timing of exposure is critical in assessing the potential for adverse effects to wildlife. Reproduction and early development are particularly sensitive life stages due to the stressed

condition of adults and the rapid growth and differentiation occurring within the embryo. For many species, exposures of a few hours to a few days during gestation and early fetal development may produce severe adverse effects. Therefore, COPEC exposures administered over a large portion of reproduction or during early development were considered to represent chronic exposures; this definition is consistent with DOI (Sample *et al.* 1996).

Uncertainty factors used to extrapolate from reported values to chronic NOAEL-equivalent RTVs are:

Extrapolation	Uncertainty Factor
Acute LD_{50} to chronic NOAEL	100
LOAEL to NOAEL	10
Subchronic to chronic	10

These endpoint-to-chronic NOAEL uncertainty factors were developed based on a review of a toxicity database of over 4000 records and were always used to lower available toxicity values to a chronic NOAEL-equivalent (i.e., a more sensitive toxicity value). These uncertainty factors are also consistent with DTSC (1996a) guidance as well as with independent review of toxicity data by other authors (Calabrese and Baldwin 1993; Sample *et al.* 1996).

1.5   ANALYSIS OF UNCERTAINTY

The use of chronic NOAEL-equivalent RTVs is likely to result in conservative assessments of impairment because environmental exposures are compared to toxicity levels at which no adverse effects were observed. Studies indicate that acute LD_{50}s derived from multiple dose toxicity tests show a high positive correlation with observed impacts in the environment (U.S. EPA 1991). DTSC (1996a, b) considers NOAELs to be 100 times more sensitive than LD_{50}s and 10 times more sensitive than LOAELs. Thus, use of chronic NOAEL-equivalent RTVs provides a substantially greater level of protection than the use of the lowest doses at which effects are observed (LOAELs) or LD_{50}s.

Nonetheless, sources of uncertainty related to use of RTVs include (1) species-to-species toxicity extrapolations, (2) laboratory-to-field toxicity extrapolations, and (3) individual-to-population level effect extrapolations.

1.5.1   Species-to-Species Toxicity Extrapolations

A source of uncertainty in this assessment is the lack of applicable wildlife species-specific toxicity data. Because of this data limitation, RTVs may be developed using available toxicity data for laboratory test species. For example, RTVs for the salt marsh harvest mouse may be developed from toxicity data for mice and rats. Studies have demonstrated that responses to toxic chemicals are a function of body size. Use of allometric adjustments to derive wildlife species-specific RTVs is consistent with available guidance (Sample *et al.* 1996; U.S. Navy 1997). Allometric equations presented in Section 1.4 were derived from empirical data. This impairment assessment assumes that allometric adjustments can be used to account for the majority of the variability observed in sensitivities to chemicals between strains of test species and wildlife species.

1.5.2   Laboratory-to-Field Toxicity Extrapolations

A number of studies (primarily for aquatic systems) have evaluated the ability of single-chemical laboratory toxicity test results to predict adverse effects of that chemical on organisms under field conditions. Preliminary chemical contaminant studies suggest that laboratory toxicity tests represent more conservative exposure scenarios than those that occur in nature (U.S. EPA 1991). Furthermore, concentrations of chemicals causing no effect in laboratory tests also do not appear to affect communities in the field. Thus, the use of chronic NOAEL-equivalent RTVs should provide a conservative level of protection to species living in the field.

1.5.3   Individual-to-Population Level Effect Extrapolations

The individual is the smallest biological "unit" that interacts directly with the environment (Suter 1993). Most toxicity data selected for the impairment assessment describe reproductive and developmental effects on individuals. Effects on individuals were then used to infer effects at the population level. Chronic reproductive impairment and abnormal development data were selected to facilitate inferences to population-level impacts (e.g., abundance, extinction). Populations are typically more resistant to stress than individuals; the loss of a few sensitive individuals is not likely to significantly affect the population (Ricklefs 1992). Therefore, inferences from effects on individuals should provide a greater level of protection to populations and communities than inferences from populations (Suter 1993).

2.0   TOXICITY PROFILE FOR COPPER

Copper is a required micronutrient for both plant and animals and is required for the proper functioning of many enzymes. In many macroinvertebrates, copper is the key component of the oxygen-carrying protein hemocyanin. Copper is readily accumulated by aquatic organisms. In fish, exposures to elevated copper may result in effects on swimming, growth, and reproduction. In mammals, copper toxicity can be greater with low dietary intake of iron, molybdenum, sulfate, and zinc; molybdenum and sulfate reduce copper absorption and enhance its excretion. The influence of these minerals is less clear with avian species.

2.1   MAMMALIAN TOXICITY - INGESTION

Seven toxicity studies examining reproductive, developmental/growth, or mortality endpoints were considered for mammals ingesting copper. The toxicity benchmark for mammals was based on a reproductive study on minks (Aulerich *et al.* 1982).

A dose of 12 mg[Cu]/kg-day was selected for use in deriving toxicity benchmarks for mammalian wildlife and is considered to be a chronic NOAEL because:

- The study considered exposure over 1-year during reproduction.

- No adverse effects on newborn minks (= kits) mortality, the length of gestation, and average kit weight were observed among minks administered this dose.

- Kit mortality, the length of gestation, and average kit weight can be directly related to reproductive success.

Features that support the use of this study include the fact that effects to a sensitive life stage (kits) were examined; a serial dosing regime was administered (e.g.,-control, 85.5 ppm, 110.5 ppm, 160.5 ppm, and 260.5 ppm[Cu]); 24 mink kits (12 male, 12 female) per treatment were examined; both a NOAEL and LOAEL were reported, permitting a limited characterization of a dose-response relationship; and an

independent review by the U.S. DOE (Sample *et al.* 1996) supports the use of this study to derive toxicity benchmarks for wildlife species.

2.1.1   Selected Toxicity Study

Minks were administered copper sulfate in their diet for 1 year (Aulerich *et al.* 1982). No adverse effects on number of kits whelped, average number of kits whelped per female, kit mortality, length of gestation, and average kit weight were observed among mink administered a concentration of 85.5 ppm[Cu] as copper sulfate in their diet (= 12 mg[Cu]/kg-day) (Aulerich *et al.* 1982).

> Test Spp: Mink; Body weight = 1.0 kg (U.S. EPA 1993a); Food Intake = 137 g/day (Bleavins and Aulerich 1981)
>
> Dosage: 85.5 ppm[Cu] = 85.5 mg[Cu]/kg$_{food}$
>
> NOAEL = (85.5 mg[Cu]/kg$_{food}$ • 137 g/day • 1 kg/1,000 g) / 1.0 kg = 12 mg[Cu]/kg-day

The authors stated that, for the most part, characteristics measured were within the normal range for mink. However, the authors reported that a trend toward greater kit mortality between birth and 4 weeks of age and reduced litter mass at weaning was observed with higher copper supplementation. These results suggested that higher doses of copper may have had an adverse effect on lactation (Aulerich *et al.* 1982). However, no statistical analyses of these data were evident in this study. Aulerich *et al.* (1982) concluded that the reproductive performance of mink on the longer-term copper supplementation was not adversely affected.

2.1.2   Other Related Toxicity Studies

Mice were administered copper gluconate in their drinking water from weanling to natural death (Massie and Aiello 1984). No adverse effects on the average or maximum life span were observed among mice administered concentrations of up to 1 x 10^{-3} M copper gluconate in their drinking water (= up to 13 mg[Cu]/kg-day).

> Test Spp: Mouse; Body weight = 0.025 kg (Lewis 1992); Water Intake = 5 ml/day (Lewis 1992)
>
> Dosage: Cu molecular wt = 64 g/mole; 1 x 10^{-3} M[Cu] • 64 g/mole = 64 mg[Cu]/L
>
> NOAEL = (64 mg[Cu]/L • 5 ml/day • 1 L/1,000 ml) / 0.025 kg = 13 mg[Cu]/kg-day

Although the authors stated that "all survival curves with mice should be regarded with some suspicion" due to undiagnosed disease or fighting, these authors concluded that "our results clearly show that copper in fact accelerates senescence." Reduced average (from 906 days in the control group to 776 days) and reduced maximum life span were observed among mice administered a concentration of 5 x 10^{-3} M copper gluconate in their drinking water (= 65 mg[Cu]/kg-day). The reduced average life span, observed at the highest administered dose, is unlikely to have adverse effects on reproductive success of wildlife populations. Because a reproductive study examining exposure over a significant portion of the gestation period was available, this study was not considered for use in deriving toxicity benchmarks for mammalian wildlife.

Eight-to-ten week-old mice were administered copper sulfate for 10 weeks (Pocino *et al.* 1991, as cited in U.S. Navy 1997). No effect on food consumption or body weight was observed among mice administered a dose of 27 mg[Cu]/kg-day. Several effects were reported from immune response experiments at doses less than 27 mg[Cu]/kg-day; however, immune responses were not considered ecologically relevant in inferring reproductive success (U.S. Navy 1997). Similarly, food consumption and body weight were considered to be less relevant in assessing reproductive success compared to responses examined in the selected study (e.g., kit survival). Because a long-term reproductive study

examining exposure during the gestation period was available, this study was not considered for use in deriving toxicity benchmarks for mammalian wildlife.

Pregnant mice were administered copper sulfate in their diet for 20 days during reproduction (Lecyk 1980). No mortality and no developmental abnormalities were observed among fetuses born to pregnant mice administered concentrations of up to 2,000 ppm[$CuSO_4$] in their diet (up to 96 mg[Cu]/kg-day).

> Test Spp: Mice; Body weight = 0.025 kg (Lewis 1992); Food Intake = 3 g/day (Lewis 1992)
>
> Dosage: $CuSO_4$ is 40% copper; 2,000 ppm[$CuSO_4$] • 0.40 = 800 mg[Cu]/kg$_{food}$
>
> NOAEL = (800 mg[Cu]/kg$_{food}$ • 3 g/day • 1 kg/1,000 g) / 0.025 kg = 96 mg[Cu]/kg-day

Increased mortality was observed among fetuses of female mice administered concentrations of 3,000 ppm[$CuSO_4$] or greater in their diet (144 mg[Cu]/kg-day or greater). Reduced litter size and increased developmental abnormalities were observed among fetuses of mice administered a concentration of 4,000 ppm as copper sulfate in their diet (= 192 mg[Cu]/kg-day). Although the authors report the above effects, no statistical analyses of these data were evident in this study. This study was not considered for use in deriving toxicity benchmarks for mammalian wildlife because a reproductive study examining exposures over 1 year during reproduction was available.

Rats were administered copper sulfate in their diet for 4 weeks (Boyden *et al.* 1938). No adverse effects on weight and food consumption were observed among rats administered concentrations of up to 500 ppm[Cu] as copper sulfate in their diet (up to 30 mg[Cu]/kg-day).

> Test Spp: Rat; Body weight = 0.25 kg (Lewis 1992); Food Intake = 15 g/day (Lewis 1992)
>
> Dosage: 500 ppm[Cu] = 500 mg[Cu]/kg$_{food}$
>
> NOAEL = (500 mg[Cu]/kg$_{food}$ • 15 g/day • 1 kg/1,000 g) / 0.25 kg = 30 mg[Cu]/kg-day

100 percent mortality was observed among rats administered a concentration of 4,000 ppm[Cu] as copper sulfate in their diet (= 240 mg[Cu]/kg-day). At a dietary concentration of 4,000 ppm[Cu], rats were observed to avoid their food and died of voluntary starvation (Boyden *et al.* 1938). This study was not considered for use in deriving toxicity benchmarks for mammalian wildlife because a reproductive study examining exposures over 1 year during reproduction was available.

Rats were administered copper acetate in their diet for 21 weeks (Llewellyn *et al.* 1985). No adverse musculoskeletal effects were observed among rats administered a concentration of 2,600 ppm[Cu] as copper acetate in their diet (= 156 mg[Cu]/kg-day).

> Test Spp: Rat; Body weight = 0.25 kg (Lewis 1992); Food Intake = 15 g/day (Lewis 1992)
>
> Dosage: 2600 ppm[Cu] = 2,600 mg[Cu]/kg$_{food}$
>
> NOAEL = (2,600 mg[Cu]/kg$_{food}$ • 15 g/day • 1 kg/1,000 g) / 0.25 kg = 156 mg[Cu]/kg-day

No adverse effects were observed at the only concentration administered in this study, prohibiting an evaluation and characterization of a dose-response relationship. This study was not considered for use in deriving toxicity benchmarks for mammalian wildlife because a reproductive study examining exposures over 1 year during reproduction was available in which a serial dosing regime was administered and both a NOAEL and LOAEL were reported.

Rats were administered copper in their diet for 30 days (Murthy *et al.* 1981, as cited in ATSDR 1998). No adverse neurological effects were observed among rats administered a dose of 13 mg[Cu]/kg-day in their diet. Murthy *et al.*'s (1981) original paper could not be acquired and evaluated. This study was not

considered further for use in deriving toxicity benchmarks for mammalian wildlife because a chronic reproductive study was available and acquired.

2.2   AVIAN TOXICITY - INGESTION

Seven toxicity studies examining reproductive, developmental/growth, or mortality endpoints were considered for birds ingesting copper. The toxicity benchmark for birds was based on a growth study on chicks (Mehring *et al.* 1960).

A dose of 33 mg[Cu]/kg-day was selected for use in deriving toxicity benchmarks for avian wildlife and is considered to be a chronic NOAEL because:

- The study considered exposure over 10 weeks.

- A sensitive life-stage was exposed (1-day-old chicks).

- No adverse effect on growth was observed in 1-day-old chicks administered this dose.

- Growth can be used to infer reproductive success.

Features that support the use of this study include the fact that effects to a sensitive life stage (1-day-old chicks) were examined; a serial dosing regime was administered (e.g., control, 36.8 ppm, 52 ppm, 73.5 ppm, 104 ppm, 147 ppm, 208 ppm, 294.1 ppm, 403 ppm, 570 ppm, 749 ppm, and 1,180 ppm[Cu] in diet); both a NOAEL and LOAEL were reported, permitting a limited characterization of a dose-response relationship; 20 individuals per treatment were examined; and an independent review by the U.S. DOE (Sample *et al.* 1996) supports the use of this study to derive toxicity benchmarks for wildlife species.

2.2.1   Selected Toxicity Study

One-day-old chicks were administered copper oxide in their diet for 10 weeks (Mehring *et al.* 1960). The basal diet contained 26 ppm[Cu]. No adverse effects on growth or survivorship were observed among 1-day-old chicks administered concentrations of up to 403 ppm[Cu] as copper oxide in their diet (up to 33 mg[Cu]/kg-day) (Mehring *et al.* 1960).

> Test Spp: Chicks; Body weight = 0.534 kg (mean at 5 wks; U.S. EPA 1988); Food Intake = 44 g/day (U.S. EPA 1988)
>
> Dosage: 403 ppm[Cu] in diet = 403 mg[Cu]/kg$_{food}$
>
> NOAEL = (403 mg[Cu]/ kg$_{food}$ • 44 g/day • 1 kg/1,000 g) / 0.534 kg = 33 mg[Cu]/kg-day

By Week 10, 30 percent reduction in growth and 15 percent mortality were observed among 1-day-old chicks administered a concentration of 749 ppm[Cu] as copper oxide in their diet (= 62 mg[Cu]/kg-day). The results of this study are consistent with the majority of NOAELs reported in the related toxicity studies.

2.2.2   Other Related Toxicity Studies

The Association of Avian Veterinarians has established a minimum daily requirement of 8 ppm[Cu] for passerines.

> Test Spp: Robin; Body weight = 0.079 kg (mean; U.S. EPA 1993b); Food Intake = 16 g/day (U.S. EPA 1993b)
>
> Dosage: 8 ppm[Cu] in diet = 8 mg[Cu]/kg$_{food}$
>
> Min. Daily Requirement = (8 mg[Cu]/ kg$_{food}$ • 16 g/day • 1 kg/1,000 g) / 0.079 kg = 1.7 mg[Cu]/kg-day

Thus, daily doses less than or equal to approximately 1.7 mg[Cu]/kg-day may result in copper deficiency in similarly sized passerines (i.e., body weight approximately 79 g).

Humphreys (1989) reported maximum safe dietary levels of 250 ppm[Cu] (= 21 mg[Cu]/kg-day) and 500 ppm[Cu] (= 50 mg[Cu]/kg-day) for growing chicks and turkeys, respectively. These maximum safe dietary levels were not selected as toxicity benchmarks because these values were poorly referenced in Humphrey's (1989) *Veterinary Toxicology* and no data were provided to support these values. Nonetheless, the maximum safe dietary level of copper for chicks is just less than the NOAEL reported in the selected study.

One-day-old broiler chickens were administered copper sulfate in their diet for 8 weeks (Norvell *et al.* 1975, as identified by DTSC). A basal diet containing 16 ppm[Cu] was considered to be nutritionally adequate by the authors. No copper-related effect on weight gain was observed among chicks administered dietary concentrations of up to 496 ppm[Cu] (up to 29 mg[Cu]/kg-day).

> Test Spp: Chick; Body weight = 0.702 kg (mean$_{control}$ at 4 wks, Norvell *et al.* 1975); Food Intake = 41 g/day (derived from U.S. EPA 1993b)
>
> Dosage: 496 ppm[Cu] in diet 496 mg[Cu]/kg$_{food}$
>
> NOAEL = (496 mg[Cu]/ kg$_{food}$ • 41 g/day • 1 kg/1,000 g) / 0.702 kg = 29 mg[Cu]/kg-day

A decrease in weight was observed among chicks administered a dietary concentration of 736 ppm[Cu] (= 43 mg[Cu]/kg-day). The reported NOAEL of this 8-week study is comparable to the selected 10-week study and supports the characterization of the NOAEL at 33 mg[Cu]/kg-day. This study was not considered for use in deriving toxicity benchmarks for avian wildlife because a longer-term study with a more detailed dosing regime was available.

Broiler cockerels were administered cupric sulfate pentahydrate for 42 days (Bakalli *et al.* 1995, as cited in U.S. Navy 1997). Increased body weight and decreased cholesterol were observed among chickens administered a dose of 22 mg[Cu]/kg-day. In a similar study, Cobb chicks were administered cupric sulfate in their diet for 4 weeks (Jensen and Maurice 1978, as cited in U.S. Navy 1997). Decreased body weight was observed among chickens administered a dose of 26 mg[Cu]/kg-day; no effects on feed to weight gain ratio, or the gizzard were observed at this dose. Gizzard erosion was observed among chickens administered a dose of 52 mg[Cu]/kg-day. These studies were not considered for use in deriving toxicity benchmarks for avian wildlife because a longer-term similar study was available.

Reduced growth was observed among chicks administered a concentration of 324 ppm[Cu] in their diet (= 27 mg[Cu]/kg-day); increased mortality was observed among chicks administered a concentration of 1,270 ppm[Cu] in their diet (= 103 mg[Cu]/kg-day) (Mayo *et al.* 1956, as cited in ATSDR 1997). No adverse effect on body weight was observed among chicks administered concentrations of up to 666 ppm[Cu] as copper sulfate in their drinking water (Underwood *et al.* 1956, as cited in ATSDR 1997). No ill effects up to the age of 8 weeks were observed among chicks administered concentrations of up to 500 ppm[Cu] in their diet (up to 41 mg[Cu]/kg-day) (Arthur *et al.* 1958, as cited in ATSDR 1997). Mayo *et al.* (1956, as cited in ATSDR 1997), Underwood *et al.* (1956, as cited in ATSDR 1997), and Arthur *et al.*'s (1958, as cited in ATSDR 1997) original papers could not be acquired and evaluated. These studies were not considered further for use in deriving toxicity benchmarks for avian wildlife because a longer-term study was available and acquired.

3.0   TOXICITY PROFILE FOR NICKEL

Nickel is an essential micronutrient and is typically found in low concentrations in animal tissue (Hoar 1975). Nickel compounds can be grouped according to their solubility in water: soluble compounds (e.g., nickel chloride, nickel sulfate, nickel nitrate) and insoluble compounds (e.g., nickel oxide). Both the soluble and insoluble nickel compounds are important with regard to all relevant routes of exposure. Generally, the soluble compounds are considered more toxic than the insoluble compounds. Ingestion of nickel compounds may cause intestinal disorders, convulsions, and asphyxia (Lewis 1992).

3.1   MAMMALIAN TOXICITY - INGESTION

Six toxicity studies examining reproductive, developmental, or growth endpoints were considered for mammals ingesting nickel. The data for adverse reproductive effects do not demonstrate consistent dose-response relationships.   The toxicity benchmark for mammals was based on a reproductive study on rats (Ambrose *et al.* 1976).

A dose of 15 mg[Ni]/kg-day was selected for use in deriving toxicity benchmarks for mammalian wildlife and is considered to be an ecologically relevant chronic NOAEL because:

- The study considered exposure over three generations.

- No adverse effects on fertility, gestation, offspring viability, and lactation indices were observed among rats administered this dose.

- Observed effects on the incidence of stillborns were considered to be transitory since no effects on stillborns were observed in subsequent generations.

- Fertility, gestation, offspring viability, and lactation were assessed and can be related to the fitness of individuals or the persistence of populations.

Features that support the use of this study include the fact that a serial dosing regime was administered (e.g.,-control, 250 ppm, 500 ppm, and 1,000 ppm[Ni] in diet); both a NOAEL and LOAEL were reported, permitting a limited characterization of dose-response relationship; 17 to 20 females per treatment in each generation were examined, and 89 to 211 weanling rats per treatment were examined; and an independent review by the U.S. DOE (Sample *et al.* 1996) supports the use of this study to derive toxicity benchmarks for wildlife species.

3.1.1   Selected Toxicity Study

Rats were administered nickel sulfate hexahydrate in their diet for three generations (Ambrose *et al.* 1976). No adverse effects on fertility (pregnancies/matings), gestation (litters cast/pregnancies), offspring viability (live pups at Day 5/live pups born), and lactation (weaned/live pups at Day 5) indices were observed among rats administered concentrations of up to 1000 ppm[Ni] as nickel sulfate hexahydrate in their diet (up to 60 mg[Ni]/kg/d).

> Test Spp: Rat; Body weight = 0.25 kg (Lewis 1992); Food Intake = 15 g/day (Lewis 1992)
>
> Dosage: 1000 ppm[Ni] in food = 1000 mg[Ni]/kg$_{food}$
>
> NOAEL = (1000 mg[Ni]/kg$_{food}$ • 15 g/day • 1 kg/1,000 g) / 0.25 kg = 60 mg[Ni]/kg-day

A higher incidence of stillborns was observed only in the first generation among rats administered concentrations of 250 ppm[Ni] or greater as nickel sulfate hexahydrate in their diet (=15mg[Ni]/kg-day or greater). This effect was considered to be transitory since no effect on the incidence of stillborns was observed in second or third generations (Ambrose *et al.* 1976). Reduced weight was observed among

weanlings born from rats administered a concentration of 1,000 ppm[Ni] as nickel sulfate hexahydrate in their diet (= 60 mg[Ni]/kg-day); however, from weanling to mating, offspring recovered from this deficit, averaging 92 percent of controls.

A dose of 15 mg[Ni]/kg-day was selected as the NOAEL-equivalent toxicity benchmark since (1) observed effects on the incidence of stillborns was transitory, (2) prior to mating, offspring recovered from differences in weight, and (3) this dose is consistent with other related studies, especially the RTI (1987) study.

3.1.2   Other Related Toxicity Studies

Male and female rats were administered nickel chloride in their drinking water for 90 days prior to breeding for 2 generations (RTI 1987, as cited in IRIS 1999). The number of live pups/litter was significantly decreased, pup mortality was significantly increased, and average pup body weight was significantly decreased in comparison with controls among F_{1a} generation (postnatal Days 1-4) rats at a dietary concentration of 500 ppm[Ni] (= 52 mg[Ni]/kg-day as estimated by IRIS 1999). Similar effects were seen with F_{1b} litters of F_o dams exposed to 500 ppm[Ni]. Increased pup mortality and decreased live litter size was observed in the F_{1b} litters of dams exposed to 50 and 250 ppm[Ni]. However, the effects observed in F_{1b} litters of dams exposed to 50 and 250 ppm[Ni] cannot be attributed to nickel because the room temperature tended to be $10°$ F higher than normal at certain times (gestation-postnatal days) and much lower in humidity; Edwards (1986, as cited in IRIS 1999) has reported that temperatures that are $10°F$ above normal during fetal development cause adverse effects. F_{1b} males and females were randomly mated on postnatal Day 70 and their offspring (F_{2a} and F_{2b}) were evaluated through postnatal Day 21 (RTI 1987, as cited in IRIS 1999). Significant body weight depression of both mothers and pups, and increased neonatal mortality during the postnatal development period were observed among rats exposed to a concentration of 500ppm[Ni]. The concentration of 250 ppm[Ni] (= 31 mg[Ni]/kg-day as estimated by IRIS 1999) produced transient depression of maternal weight gain and water intake during gestation of the F_{2b} litters. The reported incidence of short ribs in the 50 ppm[Ni] group is not considered to be attributed to nickel exposures since this effect was not seen in both the higher dose groups (IRIS 1999). This comparable reproductive study supports the selection of 15 mg[Ni]/kg-day as the NOAEL-equivalent toxicity benchmark.

Female rats were administered nickel chloride in their drinking water for 4 months (Smith *et al.* 1993, as identified by DTSC). No effects on mating success, rate of impregnation, litter size, or gestation in any generation were observed among rats administered a dose of 1.3 mg[Ni]/kg-day. A slight increase in pup mortality (4% compared to 1% in control group) shortly after birth (postnatal Day 1) in the second litter was observed among rats administered a dose of 1.3 mg[Ni]/kg-day. This minor increase (3% increase compared to controls) was not considered to be an ecologically significant adverse effect. Furthermore, this minor difference was transient since no differences in pup mortality (compared to controls) were observed on postnatal Day 21 among rats administered doses of up to 6.8 mg[Ni]/kg-day. Increased pups born dead or dying shortly after birth (postnatal Day 1) and on postnatal Day 21 were observed among rats administered a dose of 32 mg[Ni]/kg-day. US EPA (IRIS 1999) concluded that "it is hard to define a NOAEL and LOAEL" from the Smith *et al.* (1993) study "due to the lack of a clear dose-response trend at the lower doses". This study was not considered for use in deriving toxicity benchmarks for mammalian wildlife because of the lack of a clear dose-response relationship (IRIS 1999) and a reproductive study that had longer exposures and reported both a NOAEL and a LOAEL was available.

Rats were administered nickel sulfate in their drinking water for 6 months (Vyskocil *et al.* 1994, as cited in U.S. Navy 1997). No effects on weight gain were reported at a dose of 6.9 mg[Ni]/kg-day. Increased kidney weight and nephrotoxicity were observed at this dose. Increased kidney weight and nephrotoxicity were considered to be of limited ecological relevance compared to fertility, gestation,

offspring viability, and lactation in evaluating potential impacts to reproductive success. This study was not used to derive toxicity benchmarks for mammalian wildlife because a multi-generational reproductive/developmental study that examined more relevant responses was available.

Rats were administered nickel in their drinking water for three generations (Schroeder and Mitchener 1971). Increased death of young in all generations (17 deaths of young compared to 11 deaths of young in control) and number of runts in the first generation were observed among rats administered a concentration of 5 ppm[Ni] in their drinking water (= 0.5 mg[Ni]/kg-day).

> Test Spp: Rat; Body weight = 0.25 kg (Lewis 1992); Water Intake = 25 ml/day (Lewis 1992)
>
> Dosage: 5 ppm[Ni] in water = 5 mg[Ni]/L
>
> Effects Dose = (5 mg[Ni]/L • 25 ml/day • 1 L/1,000 ml) / 0.25 kg = 0.5 mg[Ni]/kg-day

The major weakness of this study is that only five pairs of rats per treatment were examined (IRIS 1999). The matings were not randomized and the males were not rotated. Interactions of nickel with other trace metals (chromium was estimated as inadequate) may have contributed to the toxicity of nickel (IRIS 1999). Furthermore, only one concentration was administered in this study, prohibiting an evaluation and characterization of a dose-response relationship. This study was not considered for use in deriving toxicity benchmarks for mammalian wildlife because a more extensive chronic reproductive study is available in which a serial dosing regime was administered and a larger sample size was examined.

Female mice were administered nickel sulfate for 180 days (Dieter *et al.* 1988, as cited in U.S. Navy 1997). Reduced body weight was reported at a dose of 396 mg[Ni]/kg-day. This effect was observed at significantly higher doses compared to other studies. Because results are not consistent with other reviewed studies and a multi-generation reproductive/developmental study was available, this study was not used to derive toxicity benchmarks for mammalian wildlife.

3.2   AVIAN TOXICITY - INGESTION

Five toxicity studies examining growth/survivorship and scatological endpoints were considered for birds ingesting nickel. The toxicity benchmark for birds was based on this growth and survivorship study on mallards (Cain and Pafford 1981).

A dose of 18 mg[Ni]/kg-day was selected for use in deriving toxicity benchmarks for avian wildlife and is considered to be an ecologically relevant chronic NOAEL because:

- The study considered exposure over 90 days during a critical and sensitive developmental period (i.e., from hatching to 90 days of age [through fledging]).

- No adverse effects on survivorship and growth were observed among mallard ducklings administered this dose.

- Growth and survivorship were assessed and can be used to infer reproductive success.

Features that support the use of this study include the fact that effects to a sensitive life stage (ducklings) were examined; a serial dosing regime was administered (e.g., control, 176 ppm, 774 ppm, and 1,069 ppm[Ni] in diet); both a NOAEL and LOAEL were reported, permitting a limited characterization of dose-response relationship; 36 individuals per treatment were examined; and an independent review by the U.S. Navy (1997) and U.S. DOE (Sample *et al.*1996) support the use of this study to derive toxicity benchmarks for wildlife species.

3.2.1   Selected Toxicity Study

One-day-old mallard ducklings were administered nickel sulfate in their diet for 90 days (from Day 1 to Day 90 of age) (Cain and Pafford 1981). No tremors or adverse effects on survivorship and growth were observed among 1-day-old mallard ducklings administered a dietary concentration of up to 176 ppm[Ni] as nickel sulfate in their diet (= 18 mg[Ni]/kg-day).

Reduced humerus weight:length ratios occurred in ducklings administered 774 ppm[Ni] (= 77 mg[Ni]/kg-day) at Day 60, but was not observed at Day 90. This transient change was considered to be of limited ecological relevance; survivorship and reduction in body weight were considered to be more relevant in evaluating potential impacts to reproductive success.

Edema in toe and leg joints were observed mallards administered a concentration of 774 ppm[Ni] (= 77 mg[Ni]/kg-day) (Cain and Pafford 1981, as indicated by DTSC). Edema in toe and leg joints was considered to be of limited ecological relevance; survivorship and reduction in body weight were considered to be more relevant in evaluating potential impacts to reproductive success.

Mallard duckling administered a concentration of 774 ppm[Ni] as nickel sulfate in their diet (= 77 mg[Ni]/kg-day) began to tremor at four weeks of age. Tremors and signs of paresis after 14 days, and 71 percent mortality within 60 days were observed among mallard ducklings administered a concentration of 1,069 ppm[Ni] as nickel sulfate in their diet (= 107 mg[Ni]/kg-day). The authors only discussed the consequences of paresis and ataxia for mallards administered a dietary concentration of 1,069 ppm[Ni]—however, the author stated that ducklings fed diets containing at least 800 ppm[Ni] would be adversely affected.

3.2.2   Other Related Toxicity Studies

Twenty-month-old mallards were administered nickel sulfate in their diet for 90 days (Eastin and O'Shea 1981, as cited in U.S. Navy 1997). Black, tarry feces were observed among mallards administered a dose of 121 mg[Ni]/kg-day. Black, tarry feces was considered to be of limited ecological relevance in evaluating potential impacts to reproductive success. This study was not considered for use in developing toxicity benchmarks for birds because a study with more relevant endpoints was available.

Broiler chicks were administered nickel sulfate in their diet for 4 weeks (NAS 1975, Nielsen 1977, Weber and Reid 1968, as cited in Eisler 1998). Normal growth was observed among chicks administered a dietary concentration of 500 mg[Ni]/kg (= 54 mg[Ni]/kg-day).

> Test Spp: Chick;   Body weight = 0.12 kg (mean at 14 day; US EPA 1988);
>
> Ingestion = 13 g_{food}/day (derived from US EPA 1988)
>
> Dosage: 500 ppm[Ni] in diet = 500 mg[Ni]/kg_{food}
>
> NOAEL = (500 mg[Ni]/kg_{food} • 13 g/day • 1 kg/1,000 g) / 0.12 kg = 54 mg[Ni]/kg-day

Marked reduction in growth was observed among chicks administered a dietary concentration of 900 or 1,300 mg[Ni]/kg (= 98 or 141 mg[Ni]/kg-day). This study was not considered for use in developing toxicity benchmarks for birds because a study with a longer exposure duration was available.

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Appendix D
Individual Species Toxicity
Test Summary

INDIVIDUAL SPECIES TOXICITY TEST SUMMARY

Species #	PotentialIndicator Species	Endpoint	Stressor	Matrix	Result	Tested for SFB/SSFB	Tested for Other	Reference
1	Diatom, *Ditylum brightwellii*	Growth Inhibition)	Cu	culture media	LOEC > 12.7 ppb		✔	35
2	Diatom, *Skeletonema costatum*	Growth Inhibition	Cu	culture media	NOEC = 25.4 ppb LOEC = 31.8 ppb		✔	29
3	Diatom, *Skeletonema costatum*	Growth Inhibition	Cu	culture media	14-d EC50 = 50 ppb		✔	12
4	Diatom, *Nitzschia thermalis*	Growth Inhibition	Cu	culture media	LOEC = 38.1 ppb		✔	29
5	Diatom, *Nitzschia closterium*	Growth Inhibition	Cu	unenriched seawater	EC50 = 10 ppb		✔	44
6	Diatom, *Nitzschia closterium*	Growth Inhibition	Cu	Nutrient enriched seawater	EC50 > 200 ppb		✔	44
7	Diatom, *Nitzschia closterium*	Growth Inhibition	Cu	culture media	EC50 = 33 ppb		✔	37
8	Diatom, *Phaeodactylum tricornutum*	Growth Inhibition	Cu	Natural seawater, 0.45µm and carbon filtered, autoclaved @ 120C (no nutrients)	EC50 = 100 ppb EC100 = 1000 ppb		✔	5
9	Diatom, *Phaeodactylum tricornutum*	4-hr photosynthetic rate(µg C l⁻¹h⁻¹)	Cu	Natural seawater, 0.45µm and carbon filtered, autoclaved @ 120C (no nutrients)	EC50 = 1000 ppb		✔	5
10	Micro-alga, *Dunaliella tertiolecta*	Growth Inhibition	Cu	Natural seawater, 0.45µm filtered, nutrients, and autoclaved @ 120C	NOEC = 8000 ppb LOEC = 12000 ppb		✔	1
11	Diatom, *Thalassiosira pseudonana*	Growth Inhibition	Cu	culture water	72-h EC50 = 5 ppb		✔	13
12	Diatom, *Thalassiosira pseudonana*	Growth Inhibition	Cu	0.45 µm filtered South Bay water + nutrients	ChrV = 14.8 ppb	✔		43
13	Diatom, *Thalassiosira pseudonana*	Growth Inhibition	Cu	0.45 µm filtered South Bay water + nutrients	ChrV = 27.8 ppb	✔		43
14	Diatom, *Thalassiosira pseudonana*	Growth Inhibition	Cu	0.45 µm filtered South Bay water + nutrients	ChrV = 24.8 ppb	✔		43
15	Diatom, *Thalassiosira pseudonana*	Growth Inhibition	Cu	0.45 µm filtered South Bay water + nutrients	ChrV = 35.8 ppb	✔		43
16	Diatom, *Thalassiosira aestevallis*	Growth Inhibition	Cu	culture water	EC50 = 19 ppb		✔	17
17	Alga, *Prorocentrans micans*	Growth Inhibition	Cu	culture water	5-d EC50 = 10 ppb		✔	39
18	Alga, *Chlorella stigmatophora*	Cell volume	Cu	culture water	21-d EC50 = 70 ppb		✔	4
19	Kelp, *Macrocystis pyrifera*	96-hr Photosynthesis inactivation	Cu	culture water	EC50 = 100 ppb		✔	8

INDIVIDUAL SPECIES TOXICITY TEST SUMMARY

Species #	PotentialIndicator Species	Endpoint	Stressor	Matrix	Result	Tested for SFB/SSFB	Other	Reference
20	Alga, *Amphidinium carteri*	Growth Inhibition	Cu	culture water	14-d EC50 < 50 ppb		✓	12
21	Alga, *Olithodiscus luteus*	Growth Inhibition	Cu	culture water	14-d EC50 > 50 ppb		✓	12
22	Alga, *Scrippsiella faeroense*	Growth Inhibition	Cu	culture water	5-d EC50 = 5 ppb		✓	39
23	Alga, *Gymnodinium splendens*	Growth Inhibition	Cu	culture water	5-d EC50 = 20 ppb		✓	39
24	Red Alga, *Champia parvula*	Reduced tetra-sporophyte growth	Cu	culture water	EC50 = 4.6 ppb		✓	45
25	Red Alga, *Champia parvula*	Reduced tetra-sporophyte production	Cu	culture water	EC50 = 13.3 ppb		✓	45
26	Red Alga, *Champia parvula*	Reduced female growth	Cu	culture water	EC50 = 4.7 ppb		✓	45
27	Red Alga, *Champia parvula*	Stopped sexual reproduction	Cu	culture water	EC50 = 7.3 ppb		✓	45
28	Alga, *Asterionella japonica*	Growth Inhibition	Cu	culture water	EC50 = 12.7 ppb		✓	14

INDIVIDUAL SPECIES TOXICITY TEST SUMMARY

Species #	PotentialIndicator Species	Endpoint	Stressor	Matrix	Result	Tested for SFB/SSFB	Tested for Other	Reference
29	**Kelp,** *Macrocystus pyrifera*	**Reduction in photosynthesis**	**Ni**	**culture water**	**EC50 = 2000 ppb**		✔	**8**
30	Brown alga, *Isochrysis galbana*	Growth Inhibition	Ni	culture water	2-d LOEC = 500 ppb		✔	54
31	Brown alga, *Isochrysis galbana*	Growth Inhibition	Ni	culture water	9-d LOEC = 80 ppb		✔	54
32	**Diatom,** *Thalassiosira pseudonana*	**Growth Inhibition**	**Ni**	**culture water: 14 ppt @ 12 C**	**2-d EC65 = 100 ppb**		✔	**54**
33	**Diatom,** *Thalassiosira pseudonana*	**Growth Inhibition**	**Ni**	**culture water: 14 ppt @ 16 C**	**2-d EC65 = 31 ppb**		✔	**54**
34	**Diatom,** *Thalassiosira pseudonana*	**Growth Inhibition**	**Ni**	**Culture water: 14 ppt @ 20 C**	**2-d EC65 = 28 ppb**		✔	**54**
35	**Diatom,** *Thalassiosira pseudonana*	**Growth Inhibition**	**Ni**	**Culture water: 14 ppt @ 24 C**	**2-d EC65 = 17 ppb**		✔	**54**
36	**Diatom,** *Thalassiosira pseudonana*	**Growth Inhibition**	**Ni**	**Culture water: 14 ppt @ 28 C**	**2-d EC65 = 80 ppb**		✔	**54**
37	**Diatom,** *Thalassiosira pseudonana*	**Growth Inhibition**	**Ni**	**Culture water: 28 ppt @ 12 C**	**2-d EC65 = 72 ppb**		✔	**54**
38	**Diatom,** *Thalassiosira pseudonana*	**Growth Inhibition**	**Ni**	**Culture water: 28 ppt @ 16 C**	**2-d EC65 =140 ppb**		✔	**54**
39	**Diatom,** *Thalassiosira pseudonana*	**Growth Inhibition**	**Ni**	**Culture water: 28 ppt @ 20 C**	**2-d EC65 =30 ppb**		✔	**54**
40	**Diatom,** *Thalassiosira pseudonana*	**Growth Inhibition**	**Ni**	**Culture water: 28 ppt @ 24 C**	**2-d EC65 =21 ppb**		✔	**54**
41	**Diatom,** *Thalassiosira pseudonana*	**Growth Inhibition**	**Ni**	**Culture water: 28 ppt @ 28 C**	**2-d EC65 =18 ppb**		✔	**54**
42	**Diatom,** *Thalassiosira pseudonana*	**Growth Inhibition**	**Ni**	**Culture water: 28 ppt**	**2-d EC65 =100 ppb**		✔	**54**
43	Dinoflagellate, *Glenodinium halli*	Growth Inhibition	Ni	culture water: 28 ppt	5-d LOEC = 50 ppb		✔	54
44	Dinoflagellate, *Glenodinium halli*	Growth Inhibition	Ni	culture water: 28 ppt	2-d LOEC = 200 ppb		✔	54
45	Dinoflagellate, *Gymnodinium splendens*	Growth Inhibition	Ni	culture water: 28 ppt @ 16 C	2-d EC65 = 1000 ppb		✔	54
46	Dinoflagellate, *Gymnodinium splendens*	Growth Inhibition	Ni	culture water: 28 ppt @ 20 C	2-d EC65 = 950 ppb		✔	54
47	Dinoflagellate, *Gymnodinium splendens*	Growth Inhibition	Ni	culture water: 28 ppt @ 24 C	2-d EC65 = 560 ppb		✔	54
48	Dinoflagellate, *Gymnodinium splendens*	Growth Inhibition	Ni	culture water: 28 ppt @ 28 C	2-d EC65 = 130 ppb		✔	54
49	Dinoflagellate, *Gymnodinium splendens*	Growth Inhibition	Ni	culture water: 28 ppt @ 30 C	2-d EC65 = 1800 ppb		✔	54
50	Dinoflagellate, *Gymnodinium splendens*	Growth Inhibition	Ni	culture water: 14 ppt @ 16 C	2-d EC65 = 1800 ppb		✔	54
51	Dinoflagellate, *Gymnodinium splendens*	Growth Inhibition	Ni	culture water: 14 ppt @ 30 C	2-d EC65 = 400 ppb		✔	54
52	Dinoflagellate, *Gymnodinium splendens*	Growth Inhibition	Ni	culture water: 28 ppt	2-d LOEC = 200 ppb		✔	54

INDIVIDUAL SPECIES TOXICITY TEST SUMMARY

Species #	PotentialIndicator Species	Endpoint	Stressor	Matrix	Result	Tested for SFB/SSFB	Tested for Other	Reference
53	Bay Mussel, *Mytilus edulis*	Embryo Development	Cu	lab water	48-h EC50 = 12.5 ppb		✔	38
54	Bay Mussel, *Mytilus edulis*	Embryo Development	Cu	lab water	48-h EC50 = 14.1 ppb		✔	38
55	Bay Mussel, *Mytilus edulis*	Embryo Development	Cu	lab water	48-h EC50 = 11.3 ppb		✔	38
56	Bay Mussel, *Mytilus edulis*	Embryo Development	Cu	lab water	48-h EC50 = 11.9 ppb		✔	38
57	Bay Mussel, *Mytilus edulis*	Embryo Development	Cu	lab water	48-h EC50 = 5.79 ppb		✔	46
58	Bay Mussel, *Mytilus edulis*	Embryo Development	Cu	lab water	48-h EC50 = 8.89 ppb		✔	47
59	Bay Mussel, *Mytilus edulis*	Embryo Development	Cu	lab water	48-h EC50 = 6.28 ppb		✔	48
60	Bay Mussel, *Mytilus edulis*	Embryo Development	Cu	lab water	48-h EC50 = 7.21 ppb		✔	46
61	Bay Mussel, *Mytilus edulis*	Embryo Development	Cu	lab water	48-h EC50 = 6.40 ppb		✔	47
62	Bay Mussel, *Mytilus edulis*	Embryo Development	Cu	lab water	48-h EC50 = 5.84 ppb		✔	48
63	Bay Mussel, *Mytilus edulis*	Embryo Development	Cu	Filtered seawater	48-h EC50 = 5.8 ppb		✔	25
64	Bay Mussel, *Mytilus edulis*	Embryo Development	Cu	North Dumbarton Site Water	48-h EC50$_T$ = 25.3 ppb 48-h EC50$_D$ = 17.8 ppb	✔		6
65	Bay Mussel, *Mytilus edulis*	Embryo Development	Cu	South Dumbarton Site Water	48-h EC50$_T$ = 27.1 ppb 48-h EC50$_D$ = 18.5 ppb	✔		6
66	Bay Mussel, *Mytilus edulis*	Embryo Development	Cu	Coyote Creek Site Water	48-h EC50$_T$ = 35.7 ppb 48-h EC50$_D$ = 22.5 ppb	✔		6
67	Bay Mussel, *Mytilus edulis*	Embryo Development	Cu	South Bay Site Water	48-h EC50 = 40.2 ppb	✔		21
68	Oyster, *Crassostrea gigas*	Embryo Development	Cu	lab water	48-h EC50 = 12.1 ppb		✔	20
69	Oyster, *Crassostrea gigas*	Embryo Development	Cu	lab water	48-h EC50 = 15.8 ppb		✔	43
70	Oyster, *Crassostrea gigas*	Embryo Development	Cu	lab water	48-h EC50 = 26.7 ppb		✔	43
71	Oyster, *Crassostrea gigas*	Embryo Development	Cu	lab water	48-h EC50 = 16.2 ppb		✔	43
72	Oyster, *Crassostrea gigas*	Embryo Development	Cu	lab water	48-h EC50 = 27.0 ppb		✔	43
73	Oyster, *Crassostrea gigas*	Embryo Development	Cu	lab water	48-h EC50 = 17.5 ppb		✔	43
74	Oyster, *Crassostrea gigas*	Embryo Development	Cu	Filtered seawater	48-h EC50 = 5.3 ppb	✔		25
75	Oyster, *Crassostrea gigas*	Embryo Development	Cu	South Bay Site Water	ChrV = 36.7 ppb	✔		43

INDIVIDUAL SPECIES TOXICITY TEST SUMMARY

Species #	PotentialIndicator Species	Endpoint	Stressor	Matrix	Result	Tested for SFB/SSFB	Other	Reference
76	**Oyster, *Crassostrea gigas***	**Embryo Development**	**Cu**	**Dumbarton Site Water**	**ChrV = 21.7 ppb**	✔		**43**
77	**Oyster, *Crassostrea gigas***	**Embryo Development**	**Cu**	**South Bay Site Water**	**ChrV = 36.7 ppb**	✔		**43**
78	Clam, *Mulinia lateralis*	Embryo Development	Cu	lab water	EC50 = 21.0 ppb		✔	38
79	Clam, *Mulinia lateralis*	Embryo Development	Cu	lab water	EC50 = 19.3 ppb		✔	38
80	Clam, *Mulinia lateralis*	Embryo Development	Cu	lab water	EC50 = 14.9 ppb		✔	38
81	Clam, *Mulinia lateralis*	Embryo Development	Cu	lab water	EC50 = 17.3 ppb		✔	38
82	Clam, *Mulinia lateralis*	Embryo Development	Cu	lab water	EC50 = 16.9 ppb		✔	38
83	Clam, *Mulinia lateralis*	Embryo Development	Cu	lab water	EC50 = 17.4 ppb		✔	38
84	**Clam, *Mya arenaria***	**Embryo Development**	**Cu**	**lab water**	**EC50 = 39 ppb**		✔	**11**
85	**Red Abalone, *Haliotes refescens***	**Embryo Development**	**Cu**	**lab water**	**EC50 = 86 ppb**		✔	**24**

INDIVIDUAL SPECIES TOXICITY TEST SUMMARY

Species #	PotentialIndicator Species	Endpoint	Stressor	Matrix	Result	Tested for SFB/SSFB	Tested for Other	Reference
86	Bay Mussel, *Mytilus edulis*	Embryo Development	Ni	Filtered seawater	48-h EC50 = 891 ppb		✔	25
87	Oyster, *Crassostrea gigas*	Embryo Development	Ni	Filtered seawater	48-h EC50 = 340 ppb		✔	25
88	Red Abalone, *Haliotes rufescens*	Survival	Ni	Filtered seawater	48-h LC50 = 224 ppb	✔		49
89	Red Abalone, *Haliotes rufescens*	Embryo Development	Ni	Filtered seawater	48-h EC50 = 144 ppb	✔		49
90	Red Abalone, *Haliotes rufescens*	Metamorphosis	Ni	Filtered seawater	14-d ChrV = 48.3 ppb	✔		49

INDIVIDUAL SPECIES TOXICITY TEST SUMMARY

Species #	PotentialIndicator Species	Endpoint	Stressor	Matrix	Result	Tested for SFB/SSFB	Tested for Other	Reference
91	Polychaete, *Nereis virens*	Survival	Cu	sediment/lab water	96-h LC50 > 249 ppb		✔	34
92	Polychaete, *Nereis diversicolor*	Survival	Cu	sediment/lab water	96-h LC50 = 200 ppb		✔	19
93	Polychaete, *Nereis diversicolor*	Survival	Cu	sediment/lab water	96-h LC50 = 445 ppb		✔	19
94	Polychaete, *Nereis diversicolor*	Survival	Cu	sediment/lab water	96-h LC50 = 480 ppb		✔	19
95	Polychaete, *Nereis diversicolor*	Survival	Cu	sediment/lab water	96-h LC50 = 410 ppb		✔	19
96	Polychaete, *Nereis diversicolor*	Survival	Cu	sediment/lab water	96-h LC50 = 364 ppb		✔	19
97	**Polychaete, *Neanthes arenaceodentata***	**Survival**	**Cu**	**lab water**	**96-h LC50 = 77 ppb**		**✔**	**32**
98	**Polychaete, *Neanthes arenaceodentata***	**Survival**	**Cu**	**sediment/lab water**	**96-h LC50 = 200 ppb**		**✔**	**32**
99	**Polychaete, *Neanthes arenaceodentata***	**Survival**	**Cu**	**sediment/lab water**	**96-h LC50 = 222 ppb**		**✔**	**32b**
100	Polychaete, *Phyllodoce maculata*	Survival	Cu	sediment/lab water	96-h LC50 =120 ppb		✔	26
				Echinoderms				
101	**Urchin, *Strongylocentrotus purpuratus***	**Embryo Development**	**Cu**	**North Dumbarton Site Water**	**EC50$_T$ = 68.09 ppb** **EC50$_D$ = 32.04 ppb**	**✔**		**6**
102	**Urchin, *Strongylocentrotus purpuratus***	**Embryo Development**	**Cu**	**South Dumbarton Site Water**	**EC50$_T$ = 81.30 ppb** **EC50$_D$ = 33.50 ppb**	**✔**		**6**
103	**Urchin, *Arbacia punctulata***	**Embryo Development**	**Cu**	**lab water**	**EC50 = 21.4 ppb**		**✔**	**38**

INDIVIDUAL SPECIES TOXICITY TEST SUMMARY

Species #	PotentialIndicator Species	Endpoint	Stressor	Matrix	Result	Tested for SFB/SSFB	Tested for Other	Reference
104	Polychaete, *Capitella capitata*	Survival	Ni	sediment/lab water	GMAV > 50000 ppb SMAV > 50000 ppb		✔	53
105	**Polychaete, *Neanthes arenaceodentata***	**Survival**	**Ni**	**sediment/lab water**	**GMAV = 35000 ppb** **SMAV = 49000 ppb**		✔	**53**
106	Polychaete, *Nereis virens*	Survival	Ni	sediment/lab water	GMAV = 35000 ppb SMAV = 25000 ppb		✔	53
107	Polychaete, *Ctenodrilus serratus*	Survival	Ni	sediment/lab water	GMAV = 17000 ppb SMAV = 17000 ppb		✔	53

INDIVIDUAL SPECIES TOXICITY TEST SUMMARY

Species #	PotentialIndicator Species	Endpoint	Stressor	Matrix	Result	Tested for SFB/SSFB	Tested for Other	Reference
108	Copepod, *Tigriopus californica*	Survival	Cu	lab water	96-h LC50 = 229 ppb		✔	31
109	Copepod, *Tigriopus californica*	Survival	Cu	lab water	96-h LC50 = 76.2 ppb		✔	31
110	Copepod, *Tigriopus californica*	Survival	Cu	lab water	96-h LC50 = 19.1 ppb		✔	31
111	Copepod, *Tigriopus californica*	Survival	Cu	lab water	96-h LC50 = 159 ppb		✔	31
112	Copepod, *Tigriopus californica*	Survival	Cu	lab water	96-h LC50 = 184 ppb		✔	31
113	Copepod, *Tigriopus californica*	Survival	Cu	lab water	96-h LC50 = 261 ppb		✔	31
114	Copepod, *Tigriopus californica*	Survival	Cu	lab water	96-h LC50 = 305 ppb		✔	31
115	Copepod, *Tigriopus californica*	Survival	Cu	lab water	96-h LC50 = 375 ppb		✔	31
116	Copepod, *Tigriopus californica*	Survival	Cu	lab water	96-h LC50 = 496 ppb		✔	31
117	Copepod, *Tigriopus californica*	Survival	Cu	lab water	96-h LC50 = 413 ppb		✔	31
118	Copepod, *Tigriopus californica*	Survival	Cu	lab water	96-h LC50 = 394 ppb		✔	31
119	Copepod, *Tigriopus californica*	Survival	Cu	lab water	96-h LC50 = 394 ppb		✔	31
120	Copepod, *Tigriopus californica*	Survival	Cu	lab water	96-h LC50 = 762 ppb		✔	31
121	Copepod, *Pseudodiaptomus coronatus*	Survival	Cu	lab water	96-h LC50 = 138 ppb		✔	52
122	**Copepod, *Eurytemora affinis***	**Survival**	**Cu**	**lab water**	**96-h LC50 = 526 ppb**		**✔**	**52**
123	**Copepod, *Acartia clausi***	**Survival**	**Cu**	**lab water**	**96-h LC50 = 52 ppb**		**✔**	**52**
124	**Copepod, *Acartia tonsa***	**Survival**	**Cu**	**lab water**	**96-h LC50 = 17 ppb**		**✔**	**42**
125	**Copepod, *Acartia tonsa***	**Survival**	**Cu**	**lab water**	**96-h LC50 = 55 ppb**		**✔**	**42**
126	**Copepod, *Acartia tonsa***	**Survival**	**Cu**	**lab water**	**96-h LC50 = 31 ppb**		**✔**	**42**
127	**Mysid Shrimp, *Mysidopsis bahia***	**Survival**	**Cu**	**lab water**	**96-h LC50 = 181 ppb**		**✔**	**22**
128	**Mysid Shrimp, *Mysidopsis bahia***	**Survival**	**Cu**	**lab water**	**96-h LC50 = 164 ppb**		**✔**	**38**
129	**Mysid Shrimp, *Mysidopsis bahia***	**Survival, growth, & fecundity**	**Cu**	**lab water**	**ChrV = 54.09 ppb**		**✔**	**22**
130	Mysid Shrimp, *Mysidopsis bigelowi*	Survival	Cu	lab water	96-h LC50 = 141 ppb		✔	52
131	**Crab, *Cancer magister***	**Larval Survival**	**Cu**	**Filtered seawater**	**96-h LC50 = 49 ppb**		**✔**	**25**
132	**Crab, *Cancer magister***	**Larval Survival**	**Cu**	**Filtered seawater**	**96-h LC50 = 19.6 ppb**		**✔**	**25**
133	**Crab, Cancer maenas**	**Larval Survival**	**Cu**	**lab water**	**96-h LC50 = 600 ppb**		**✔**	**9**

INDIVIDUAL SPECIES TOXICITY TEST SUMMARY

Species #	PotentialIndicator Species	Endpoint	Stressor	Matrix	Result	Tested for SFB/SSFB	Tested for Other	Reference
134	**Crab, *Cancer magister***	**Larval Survival**	**Ni**	**Filtered seawater**	**96-h LC50 = 4260 ppb**		✔	**25**
135	**Shrimp, *Mysidopsis bahia***	**Survival**	**Ni**	**South Bay Water**	**96-h LC50 = 923 ppb**	✔		**21**
136	Shrimp, *Mysidopsis bigelowi*	Survival	Ni	lab water	96-h LC50 = 634 ppb		✔	53
137	Shrimp, *Heteromysis formosa*	Survival	Ni	lab water	96-h LC50 = 152 ppb		✔	53
138	**Copepod, *Acartia clausi***	**Survival**	**Ni**	**lab water**	**96-h LC50 = 3406 ppb**		✔	**7**
139	Copepod, *Nitocra spinipes*	Survival	Ni	lab water	96-h LC50 = 6000 ppb		✔	7
140	**Copepod, Eurytemora affinis**	**Survival**	**Ni**	**lab water**	**96-h LC50 = 11240 ppb**		✔	**7**
141	**Amphipod, Corophium volutator**	**Survival**	**Ni**	**lab water**	**96-h LC50 = 18950 ppb**		✔	**7**
142	Hermit Crab, *Pagarus longicarpus*	Survival	Ni	lab water	96-h LC50 = 47000 ppb		✔	7

INDIVIDUAL SPECIES TOXICITY TEST SUMMARY

Species #	PotentialIndicator Species	Endpoint	Stressor	Matrix	Result	Tested for SFB/SSFB	Other	Reference
143	**Minnow, *Menidia beryllina***	**Survival**	**Cu**	**South Bay Water**	**LC50 > 256.6 ppb**	✔		**21**
144	**Minnow, *Menidia beryllina***	**Survival**	**Cu**	**lab water**	**LC50 = 115.4 ppb**	✔		**46**
145	**Minnow, *Menidia beryllina***	**Survival**	**Cu**	**lab water**	**LC50 = 96.5 ppb**	✔		**47**
146	**Minnow, *Menidia beryllina***	**Survival**	**Cu**	**lab water**	**LC50 = 123.0 ppb**	✔		**48**
147	**Minnow, *Menidia beryllina***	**Survival and Growth**	**Cu**	**lab water**	**ChrV > 110 ppb**	✔		**43**
148	Minnow, *Menidia menidia*	Survival	Cu	lab water	LC50 = 66.6 ppb		✔	52
149	Minnow, *Menidia menidia*	Survival	Cu	lab water	LC50 = 216.5 ppb		✔	52
150	Minnow, *Menidia menidia*	Survival	Cu	lab water	LC50 = 101.8 ppb		✔	52
151	Minnow, *Menidia menidia*	Survival	Cu	lab water	LC50 = 97.6 ppb		✔	52
152	Minnow, *Menidia menidia*	Survival	Cu	lab water	LC50 = 155.9 ppb		✔	52
153	Minnow, *Menidia menidia*	Survival	Cu	lab water	LC50 = 197.6 ppb		✔	52
154	Minnow, *Menidia menidia*	Survival	Cu	lab water	LC50 = 190.9 ppb		✔	52
155	Minnow, *Menidia peninsulae*	Survival	Cu	lab water	LC50 = 140 ppb		✔	52
156	Minnow, *Cyprinodon variegatus*	Survival	Cu	lab water	LC50 = 368 ppb		✔	18
157	Minnow, *Cyprinodon variegatus*	Survival	Cu	lab water	LC50 = 280 ppb		✔	52
158	Mummichog, *Fundulus heteroclitus*	Survival	Cu	lab water @ 5.5 ppt	LC50 = 3100 ppb		✔	10
159	Mummichog, *Fundulus heteroclitus*	Survival	Cu	lab water @ 6.1 ppt	LC50 = 2300 ppb		✔	10
160	Mummichog, *Fundulus heteroclitus*	Survival	Cu	lab water @ 23.6 ppt	LC50 = 2000 ppb		✔	10
161	Mummichog, *Fundulus heteroclitus*	Survival	Cu	lab water @ 24 ppt	LC50 = 400 ppb		✔	10
162	**Topsmelt, *Atherinops affinis***	**Survival**	**Cu**	**lab water**	**LC50 = 288 ppb**		✔	**2**
163	**Topsmelt, *Atherinops affinis***	**Survival**	**Cu**	**lab water**	**LC50 = 212 ppb**		✔	**2**
164	**Topsmelt, *Atherinops affinis***	**Survival**	**Cu**	**lab water**	**LC50 = 235 ppb**		✔	**2**

INDIVIDUAL SPECIES TOXICITY TEST SUMMARY

Species #	PotentialIndicator Species	Endpoint	Stressor	Matrix	Result	Tested for SFB/SSFB	Tested for Other	Reference
165	Pompano, *Trochinotus caroli nas*	Survival	Cu	lab water	LC50 = 360 ppb		✔	3
166	Pompano, *Trochinotus caroli nas*	Survival	Cu	lab water	LC50 = 380 ppb		✔	3
167	Pompano, *Trochinotus caroli nas*	Survival	Cu	lab water	LC50 = 510 ppb		✔	3
168	**Summer Flounder, *Paralichthys dentatus***	**Early embryo cleavage**	**Cu**	**lab water**	**LC50 = 16.3 ppb**		**✔**	**52**
169	**Summer Flounder, *Paralichthys dentatus***	**Early embryo cleavage**	**Cu**	**lab water**	**LC50 = 11.9 ppb**		**✔**	**52**
170	**Summer Flounder, *Paralichthys dentatus***	**Blastula stage embryo**	**Cu**	**lab water**	**LC50 = 111.8 ppb**		**✔**	**52**
171	Winter Flounder,*Pseudopleuronectes americanus*	Embryo	Cu	lab water	LC50 = 77.5 ppb		✔	52
172	Winter Flounder,*Pseudopleuronectes americanus*	Embryo	Cu	lab water	LC50 = 167.3 ppb		✔	52
173	Winter Flounder,*Pseudopleuronectes americanus*	Embryo	Cu	lab water	LC50 = 52.7 ppb		✔	52
174	Winter Flounder,*Pseudopleuronectes americanus*	Embryo	Cu	lab water	LC50 = 158.0 ppb		✔	52
175	Winter Flounder,*Pseudopleuronectes americanus*	Embryo	Cu	lab water	LC50 = 173.7 ppb		✔	52
176	Winter Flounder,*Pseudopleuronectes americanus*	Embryo	Cu	lab water	LC50 = 271.0 ppb		✔	52
177	Winter Flounder,*Pseudopleuronectes americanus*	Embryo	Cu	lab water	LC50 = 132.8 ppb		✔	52
178	Winter Flounder,*Pseudopleuronectes americanus*	Embryo	Cu	lab water	LC50 = 148.2 ppb		✔	52
179	Winter Flounder,*Pseudopleuronectes americanus*	Embryo	Cu	lab water	LC50 = 98.2 ppb		✔	52

INDIVIDUAL SPECIES TOXICITY TEST SUMMARY

Species #	PotentialIndicator Species	Endpoint	Stressor	Matrix	Result	Tested for SFB/SSFB	Other	Reference
180	**Minnow, *Menidia beryllina***	**Survival**	**Ni**	**South Bay Water**	**LC50 = 20519 ppb**	✔		**21**
181	Minnow, *Menidia menidia*	Survival	Ni	lab water	LC50 = 7958 ppb		✔	7
182	Minnow, *Menidia peninsulae*	Survival	Ni	lab water	LC50 = 38000 ppb		✔	7
183	Mummichog, *Fundulus heteroclitus*	Survival	Ni	lab water	LC50 = 149900 ppb		✔	7
184	**Striped Bass, *Marone saxatilis***	**Survival**	**Ni**	**lab water**	**LC50 = 21000 ppb**		✔	**7**
185	**Topsmelt, *Atherinops affinis***	**Survival**	**Ni**	**Filtered seawater**	**LC50 = 26550 ppb**	✔		**49**
186	**Topsmelt, *Atherinops affinis***	**Survival and Growth**	**Ni**	**Filtered seawater**	**ChrV = 4230 ppb**	✔		**49**

Key:
Native Species = Red
Native Genera = Blue
Common Test Species = Green

Individual Species Toxicity Summary References

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Appendix E
Assumptions of the AERAP
Statistical Model

APPENDIX E
ASSUMPTIONS OF THE AERAP STATISTICAL MODEL

The AERAP model is a statistical model, based on a linear regression between Logit(p), the logit of percentage species affected (e.g., species for which the LC_{50} is exceeded), and x, the natural logarithm of exposure concentration, where Logit(p) = Ln(p/(1-p)). The model takes the form

$$Logit(p) = a + b \cdot x$$

where a and b are regression coefficients.

This type of linear model is termed a (log-)logistic, logit, or log-odds model, and is often used to represent toxicity versus concentration relationships in toxicity tests. Aldenberg and Slob (1993) demonstrated that NOEC data across multiple species are well described by log-logistic models.

In fact, theoretical considerations suggest that the best representation of LC_{50} data should be obtained by using a probit transformation, in which case the left hand side of the regression model is given by Probit(p) = $F^{-1}(p)$, where F^{-1} is the inverse of the cumulative normal distribution (Stephan, 1977; Bartell et al., 1992). A probit model assumes that the critical value of concentration that will cause a lethal response in an individual test animal is a normally distributed random variable, so that the probability that this critical value is less than (or equal to) the actual exposure concentration can be computed from the cumulative normal probability function (Finney, 1964). Unfortunately, the probit model is difficult to use, as it requires the calculation of the inverse cumulative normal function. The logit model is used as a simpler substitute for the probit model because the cumulative logistic probability function provides a close approximation of the cumulative normal function, but is much easier to compute. The only difference between the logistic and probit formulations is that the logistic has slightly fatter tails (Pindyck and Rubinfeld, 1981). Indeed, the logistic distribution closely resembles the Student t distribution with 7 degrees of freedom (Hanushek and Jackson, 1977). The fact that the logistic model has slightly fatter tails than the cumulative normal model is a conservative assumption for the AERAP: that is, the logistic model will tend to estimate a higher percentage of genera effected for concentrations near the lower limit of reported LC_{50}s across all species than would be obtained from use of a probit model.

The basic statistical assumption of the AERAP model is that a linear relationship exists between the natural logarithm of exposure concentration and the logit of the cumulative percent of species affected. A linear logistic model between the natural logarithm of exposure concentration and the logit of the cumulative percent of species exceeding the LC_{50} in laboratory toxicity tests is well supported by the available data. The additional general assumptions required for application of the model to natural communities are the following (Parkhurst et al., 1996):

- As exposure concentration increases, the number of species affected also increases.

- The relationship between exposure concentration and effects "on the community of species" can be estimated from the laboratory toxicity tests reported in EPA water quality criteria documents.

- The logistic regression developed from laboratory LC_{50} data is representative of effects in natural communities.

- There are no confounding effects of habitat, water quality, bioavailability, and species (such as competition and predation) that alter the extrapolation from laboratory to field results.

Because the AERAP logistic model is a classical linear regression model, the key statistical assumptions of the AERAP, and the consequences of their violation, are similar to those found for all regression models. Kennedy (1979) lists the five basic assumptions of the linear regression model as follows:

1. *The dependent variable is a linear function of a specific set of independent variables, plus a disturban*ce. This assumption is violated when the relationship is non-linear, or the set of regressors is incorrect. A linear relationship between the natural log of exposure concentration and effect is well accepted (Aldenberg and Slob, 1993), and is borne out by the fit of the regression models presented by Parkhurst et al. (1996). Potential errors in specification of variables has long been a focus in laboratory toxicity testing (i.e., is the observed response due to some other condition of the test than the exposure concentration?) and is resolved as far as possible through use of established testing procedure protocols. Incorrect regressors is a bigger concern for the extrapolation from laboratory to field conditions, where toxicity may be determined by factors other than exposure concentration. For the South San Francisco Bay, this issue is resolved through use of a site-specific WER.

2. *The expected value of the disturbance term is zero*. The linear model assumes that the expected value of disturbances about the regression line is zero. Non-zero mean errors occur if, for example, there are systematically positive or systematically negative errors of measurement in calculating the dependent variable. The condition is equivalent to a linear model with a biased intercept term. Non-zero mean disturbances are not expected to present a problem for the AERAP regressions. Further, predictions by the AERAP are relatively insensitive to small errors on the intercept term when converted back from a logit to a probability basis.

3. *The disturbances have uniform variance and are uncorrelated*. The sample on which the regression is based consists of results from independent toxicity tests and serial correlation should not apply. A bigger problem for many environmental applications is the assumption of uniform variance of disturbances. In particular, the variances are often found to be scale-dependent, with increasing expected magnitude of disturbances in predictions of the dependent variable as the magnitude of the independent variable increases (a condition known as heteroscedasticity). In many cases, a logarithmic transformation of the independent variable is sufficient to remove heteroscedasticity. Such is the case for the toxicity data, and this is the reason a log-logistic model was used. The log-logistic models do not violate assumptions of uniform variance.

4. *Observations on independent variables can be considered fixed in repeated samples.* The mathematical derivation of the linear model assumes that the independent variables are not themselves random (and thus can be considered fixed in repeated samples), and thus are not correlated with the disturbances. In environmental analysis, the independent variables are often stochastic. Kennedy (1979) shows that the presence of stochastic regressors does not cause any significant problems with the application of linear models, except where the regressors are contemporaneously correlated with the error term. Observations on the independent variables in the toxicity tests may be considered fixed in repeated samples. Environmental exposure concentrations are inherently stochastic, but are not expected to be contemporaneously correlated with errors in predicted response in laboratory tests.

5. *No exact linear relationships occur between independent variables, and there are more observations than independent variables.* Occurrence of relationships between independent variables is applicable only to multivariate regression, whereas the AERAP employs a univariate model. The second part of this assumption simply says (for univariate regression) that at least two observations are necessary to define a line.

In sum, all of the basic statistical assumptions of linear models appear to be met in the AERAP application. The major questions that may affect the applicability of the AERAP model are non-statistical in nature (e.g., ability to extrapolate from laboratory toxicity tests to environmental impacts).

CONFIDENCE LIMITS FOR THE AERAP MODEL

Confidence limits for the AERAP model are developed using standard equations for the linear regression model. Confusion on the part of the reviewer arises because of the logistic transformation. That is, the regression is a logistic regression, conducted in logit space. On the logit scale, the confidence limits "spread" toward the tails of the regression, as expected for the linear regression model. Back transformation to the probability scale of percent genera effected, as presented in the document, results in an apparent collapsing of these confidence limits.

These concepts are most easily demonstrated graphically. In the figure, the solid blue line is the linear regression of the logit versus natural log of concentration, and the dashed blue line is the 95% upper confidence limit. This confidence limit expands with distance from the mean concentration value of 2.3, as is expected for the linear regression model. The solid red line represents the logit model converted back to probability, while the dashed red line represents the 95% upper confidence limit on the logit model as probability. Transformation to the probability scale causes the width of the confidence bound to appear to narrow toward the tail of the distribution.
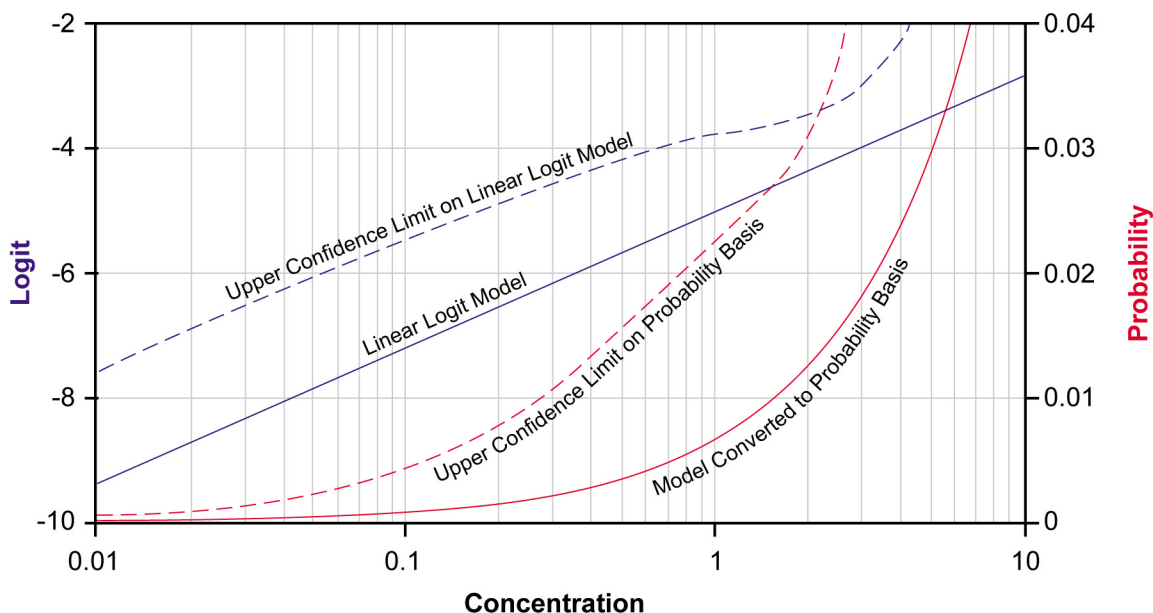
Mathematically, the equation by which the AERAP model calculates 95% prediction limits is given on p. 3-25 of the AERAP documentation (Parkhurst et al., 1996):

$$a + b \cdot x \ \pm \ \sqrt{1/n + (x - \bar{x})^2 / d}$$

where *a* and *b* are the coefficients of the logistic regression, *x* is the natural logarithm of concentration at the point of prediction, the overbar indicates the average of the natural logarithms of all observed concentrations, and

$$d = \sum (x - \bar{x})^2$$

This formula is the usual one for the confidence interval for the mean value of the response associated with an observation *x* (e.g., Wonnacott and Wonnacott, (1977).

By inspection, the size of the confidence interval increases in accordance with the distance between an observed value of *x* and the mean value of *x*, which results in expanded tails of the confidence interval. The dependent variable in the model is, however, the logit of the percent of species affected, *p*, given by

$$Logit\,(p) = Ln\left(\frac{p}{1-p}\right)$$

which means

$$p = \frac{e^{Logit(p)}}{1 + e^{Logit(p)}}$$

The derivative of p with respect to the logit goes toward zero as the logit becomes very small or very large, which occurs when p approaches 1 or 0. As a result, the wide confidence bands on Logit(p) are collapsed toward the tails when the graph is plotted with p on the y axis.

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Appendix F
AERAP Analysis Database (Acute)

AERAP Analysis Database (Acute)

Species Name	Analysis Scenario			
	National/No Plants	EPA WER Cookbook	LSSFB Resident/Surrogate	LSSFB Resident
Mytilus edulis	Y	Y	Y	Y
Paralichthys dentatus	Y	Y	Y	Y
Mulinia lateralis	Y	N	N	N
Crassostrea gigas	Y	N	Y	N
Crassostrea virginica	Y	N	Y	N
Arbacia punctulata	Y	Y	N	N
Acartia tonsa	Y	Y	Y	Y
Mya arenaria	Y	Y	Y	Y
Acartia clausi	Y	Y	N	Y
Haliotes rufescens	Y	N	Y	N
Pseudopleuronectes americanus	Y	N	Y	N
Phyllodoce maculata	Y	N	N	N
Menidia beryllina	Y	Y	Y	Y
Menidia menidia	Y	N	Y	N
Mysidopsis bigelowi	Y	Y	Y	Y
Pseudodiaptomus coronatus	Y	N	N	N
Menidia peninsulae	Y	Y	Y	Y
Neanthes arenaceodantata	Y	N	Y	N
Mysidopsis bahia	Y	N	Y	N
Nereis virens	Y	Y	Y	Y
Tigriopus californica	Y	Y	Y	Y
Atherinops affinis	Y	Y	Y	Y
Cyprinodon variegatus	Y	N	N	N
Nereis diversicolor	Y	Y	Y	Y
Trochinotus carolinas	Y	N	N	N
Eurytemora affinis	Y	Y	Y	Y
Cancer maenas	Y	N	Y	N
Fundulus heteroclitus	Y	N	N	N

Y = Used in the analysis; N = Not used in the analysis

Appendix G
TRC Review Comments
10/15/99

APPENDIX G

Report to the TMDL Work Group on the Technical Review Committee Review of the Impairment Assessment Report

DRAFT

October 15, 1999

The review of the documents produced in the calculation of total maximum daily loads (TMDLs) for copper and nickel in South San Francisco Bay by a Technical Review Committee (TRC) is an important part of the overall TMDL project plan. The purpose of the TRC review process is to establish a solid technical basis for project activities, to establish and maintain the trust and support of a wide range of interested stakeholders, and to acquire new ideas and perspectives.

The Draft Final Impairment Assessment Report was the second of the TMDL documents to be reviewed by the Technical Review Committee. The purpose of this report is to provide a record of the technical review process, present the comments of the Technical Review Committee members, to evaluate the effectiveness of this review process, and to identify the actions that are proposed in response to the Technical Review Committee's comments on the Impairment Assessment Report.

1. Meeting Summary

A Technical Review Committee (TRC) was convened on September 13, 1999 to review the *Draft Final Impairment Assessment Report* (Tetra Tech, 1999). The members of the TRC were:

> Ken Bruland, University of California at Santa Cruz
> David Hansen, HydroQual
> Jim Kuwabara, U.S. Geological Survey
> Jonathan Phinney, Center for Marine Conservation, Washington, D.C.

Resumes for the TRC members are presented in the TMDL Task 9 TRC procedures document (Tetra Tech, 1998). The process of selecting the TRC members is also described in the Task 9 report.

Two weeks prior to the September 13 meeting, the TRC members were provided with the Impairment Assessment Report and a list of questions that should be considered in their review. The information presented to the TRC prior to the meeting is included in Attachment 1. The reviewers were also provided with the Conceptual Model Report (Tetra Tech, 1999), a brief overview of the TMDL efforts underway (Attachment 2) and a copy of the TRC Procedures Document (Tetra Tech, 1998).

There were three parts to the review meeting. The first part consisted of a presentation by Tetra Tech on the Impairment Assessment Report. This presentation lead to several questions, and the graphics that were prepared for the meeting were used several times to guide the discussions. In the second part of the meeting the reviewers met to compare notes and to discuss their findings.

A question and answer session made up the third part of the meeting. The reviewers provided answers to the questions that were developed to guide the review, and the reviewers asked several questions regarding information presented in the Impairment Assessment Report.

2. Summary of Findings

The written comments provided by the TRC members are presented in Attachment 3. The following is a summary of these findings. First, the general findings on the ability of the Impairment Assessment Report to meet the overall objectives are presented. Next, the specific findings from the written comments of the reviewers are summarized. The primary objective of this portion of the summary is to confirm that the most important features of the reviewer's comments have been captured. (*This summary was also presented to the reviewers to make sure that this objective was met, and their responses are provided in Attachment 4.*) The preparation of this summary also provides a basis for identifying the required responses and modifications to the Impairment Assessment Report.

2.1 General Findings of the TRC

The reviewers found that the report was well written, complete, and scientifically sound. They generally agreed that, based on the existing information, the copper and nickel values calculated for site-specific objectives in lower South San Francisco Bay would be protective of invertebrates and fish, but may not protect phytoplankton. They agreed that additional studies are necessary to rule out the possibility of copper toxicity to phytoplankton.

2.2 TRC Response to Review Questions

A series of questions were sent to the TRC members prior to the meeting to help guide the review process. These questions were prepared by the TMDL Work Group's Subcommittee for the TRC Review. During the afternoon session of the review meeting, these questions were discussed. The reviewers also submitted written responses to these questions, along with any other comments they had concerning the report. The TRC responses to these questions are summarized below. The Tetra Tech impairment assessment team responses to the TRC comments are presented in italics following each of the reviewer comments. These responses either address each comment specifically, or indicate what changes will be made to the final report.

> **1) Is the method or approach for evaluating impairment in the report reasonable?**
>
> The reviewers agreed that the approach used in the impairment assessment was reasonable and complete based on our current understanding. It was noted that although the focus was on dissolved metals, particulate metals may also be important to fish and macroinvertebrates. The bioavailability of particulate copper and nickel to fish and macroinvertebrates is poorly understood, and should be acknowledged in the report.

The role that particulate-bound copper and nickel play in toxicity to aquatic organisms is one that is not very well understood. While there is a modest amount of information in the scientific literature that addresses this issue, several issues remain unanswered (e.g., the effects of full-life exposure to particulate-bound copper/nickel, the ability of the organism to assimilate the metal from the particle, the effects of site-specific water quality). According to John Hunt (Marine Pollution Studies Laboratory, UCSC, personal communication, October 1999), since copper and nickel toxicity is mainly caused by the ionic form of the metals (i.e., Cu^{2+} and Ni^{2+}), particulate-bound copper and nickel toxicity is very site-specific and would be dependent upon the nature of the particle. For example, copper or nickel bound to particulate clay would be more easily assimilated by an organism than would copper or nickel bound to a strong organic ligand. John Hunt also stated that the current procedure that is used to assess the effects of particulate-bound metals is the WER. The WER is used to take into consideration the site-specific water quality characteristics that affect the toxicity of particulate-bound metals.

While the Impairment Assessment team acknowledges that particulate-bound copper and nickel can impair aquatic life, it was not used as an assessment tool because of a lack of site-specific information that could be used to directly link particle-bound copper and nickel to impairment of the beneficial uses of Lower South San Francisco Bay.

2) Are all of the relevant data included or summarized adequately in the report?

In general, the reviewers found that most of the relevant data were included and were well presented in the report. However, a few studies concerning sediment toxicity issues and macroinvertebrates were not adequately addressed. Some reviewers felt that additional information may be available on the dietary doses of copper and nickel that cause toxicity problems in birds and mammals. Jonathan Phinney suggested that lipophilic organic metal complexes should also be discussed, since these complexes are very bioavailable and since industrial sources of the ligands may exist in the South Bay.

The final report will contain additional information on sediment toxicity and macroinvertebrates, as discussed in more detail in the responses to Question 3 below (SEM/AVS and Benthic Macroinvertebrate sections). Additional information will also be presented on dietary copper and nickel exposure to birds and mammals. A discussion of lipophilic organic metal complexes will be included in the phytoplankton section of the report.

3) Does the discussion of indicators reflect the current state of scientific knowledge on the relevant topics?

The reviewers identified several issues and questions concerning the discussion of indicators. These ranged from confusion with the terminology to more specific issues concerning each of the potential indicator types and whether or not they should be used to assess copper and nickel in the South Bay. These issues are summarized below.

Terminology - Some of the reviewers felt that the use of the indicator terminology in the report was confusing (e.g., indicator species vs. indicator tests vs. indicator criteria).

Indicators are defined on page 2-1 in the "Impairment Assessment Report: Draft Final" (August 30, 1999) as: " measurable quantities that are so strongly associated with particular environmental conditions that the value of the measured quantity can be used to indicate the existence and maintenance of these conditions". This definition has three main elements: the measurable quantity, the value of the quantity, and the environmental condition. The measurable quantity can be an organism, an ecological community, or measures of biogeochemical conditions. The value of the quantity is important and denotes that there can be a quantitative relationship between the indicator and the environmental condition (designated Beneficial Uses).

Three primary indicators were used in this assessment (Individual Species Toxicity Tests, AERAP, and Site-Specific Studies). They were used in the following manner:

Individual Species Toxicity Tests - This indicator utilized the toxicological responses of individual organisms listed in the National Water Quality data set to either copper or nickel. Their responses provided a measurable quantity (sensitivity endpoint), the value of which could be used to assess the potential for impairment of Beneficial Uses under specific water quality conditions (e.g., laboratory water).

AERAP - This indicator utilized the toxicological responses of individual organisms listed in the National Water Quality data set to copper, much like the Individual Species Toxicity Tests. However, this indicator was used to assess the data set on a community basis. In this case, their responses provided a measurable quantity (ERC), the value of which could be used to assess the potential for impairment of Beneficial Uses under specific water quality conditions (e.g., laboratory water).

Site-Specific Studies - This indicator utilized the toxicological responses of the most sensitive organisms listed in the National Water Quality data set to either copper or nickel in ambient Lower South San Francisco Bay water. Their responses provided a measurable quantity (sensitivity endpoint), the value of which could be used to assess the potential for impairment of Beneficial Uses under site-specific water quality conditions.

Community Analysis - Dave Hansen suggested that even though it was correct to not include community analysis as a technique for assessing copper and nickel in the South Bay, that a comprehensive discussion of the health of the South Bay ecosystem would be valuable. Community assessments could be used to help determine if copper toxicity to sensitive phytoplankton species is having a significant impact on the health of the ecosystem (if phytoplankton toxicity is demonstrated in the recommended studies).

The use of community measures to assess impairment is difficult to interpret, even with a comprehensive data set, which we do not have. Our strategy was to use the most sensitive indicators (e.g., toxicity tests) to assess potential impairment. The underlying

assumption was that by protecting the most sensitive organisms in the community, we would be protective of the community as a whole.

SEM/AVS Ratios - Several of the reviewers felt that SEM/AVS ratios in sediments together with metal concentrations in pore waters should not have been rejected as an indicator. They suggested that the USGS may have some SEM/AVS data, and that other researchers may have pore water data. Even if no SEM/AVS data have been collected in the bay, the reviewers felt that these measurements should be made since the technique should be valid (since the same processes occur in all sediments) and since significant sediment toxicity has been observed. Sediment TIEs could also be conducted to determine if copper and nickel are the cause of this toxicity.

Janet Thompson, Byeong Lee, and Michelle Hornberger of the USGS have been contacted regarding the SEM/AVS sampling that they were reported to have conducted in Lower South San Francisco Bay. To date, the project team has not received any additional information or data regarding these samples. When the information has been obtained it will be evaluated for inclusion in the final report.

We will obtain the U.S. EPA Whole-Sediment TIE Guidance Manual when it becomes available (it is currently under review), and assess its application to the impairment assessment.

Benthic Macroinvertebrates - Several of the reviewers felt that benthic macroinvertebrates should be considered as an indicator in view of the observed sediment toxicity and studies conducted by the USGS. Jonathan Phinney suggested that a benthic index developed for Chesapeake Bay may be useful to evaluate effects on the benthic community.

When the Impairment Assessment was being conducted, the Tetra Tech project team consulted with Dr. Bruce Thompson, Principal Investigator for the Regional Monitoring Program Benthic Pilot Study, regarding the use of benthic macroinvertebrates as an indicator. At that time, Dr. Thompson stated that reference ranges had not yet been completed for habitat types found in South Bay, and that all available data were being used to define reference conditions for the area. In response to TRC comments, Dr. Thompson and other local scientists were consulted to determine the status of benthic macroinvertebrate assessment information. Reference ranges for benthic macroinvertebrates for South Bay are defined in a draft publication currently under review that was produced by the San Francisco Estuary Institute: " San Francisco Estuary Regional Monitoring Program for Trace Substances, Results of the Benthic Pilot Study, 1994-1997- Volumes I and II, Identifying Benthic Responses to Contamination in San Francisco Bay"(Lowe and Thompson, 1999). The paper describes the approach and results of the reference ranges for the three major benthic assemblages that exist in the estuary: 1) Central Bay marine; 2) estuarine; and 3) fresh-brackish (each assemblage has sub-assemblages that reflect differences in salinity, sediment type, or disturbance).

All of the RMP benthic macroinvertebrate monitoring data were used in defining the reference conditions. The same data set cannot be simultaneously used to define reference conditions and to determine impairment. Therefore, these data are not available for the impairment assessment.

Dr. Janet Thompson (USGS Menlo Park) was interviewed regarding the possibility of evaluating historic data sets that have both the requisite sampling frequency and relevant contaminant information to supplement the impairment assessment. The USGS data from the Palo Alto mudflat were identified as a potential candidate data set for applying the RMP reference envelope procedures. In addition, there are other archived samples that could be worked up to provide data sets for the supplemental analysis. The decision to proceed would require the allocation of special studies funding and will need the approval of the TWG and the City of San Jose. Each candidate group of archived samples are briefly described below:

Palo Alto Mudflat Data: Replicate samples (3) were collected and analyzed at three sites near Sand Point from 1974-1985, intermittently collected through 1997, and monthly sampled from 1998-present. Samples have been collected intermittently since 1985, but processing has been minimal due to funding limitation. This data set would nicely complement the long-term data collected by Sam Luoma (USGS Palo Alto) at the same location. USGS recently started collecting data at these sites. A Masters student could undertake the analysis if funding was available. The analysis could help determine if there has been a benthic community change that is comparable to the metal contaminant change. This would be a good set of data to examine the index described in the Lowe and Thompson (1999) report referenced above.

South Bay Benthic Data (1991-1996): Replicate (3) benthic samples were collected each month at 6-13 stations from 1991-1996, with 1-5 stations being collected at or south of Dumbarton Bridge. From 1991-1996, 6-7 stations were collected with one station at Dumbarton Bridge; from 1993-1996, 13 stations were collected with 5 stations collected at or south of Dumbarton Bridge. The large bivalve species were removed and measured from all samples except during 1996. The Dumbarton Bridge Station is midchannel at the bridge. The stations collected from 1993-1996 include one at the mouth of Coyote Creek (concurrent with Luoma trace metal analysis of sediment and tissue of Macoma balthica), one in the southern channel but up the gradient from Coyote Creek (USGS historical water collection site, water data available from 1968 to present), two stations in the shallow subtidal/deep intertidal on the western flats (one near Palo Alto, and one opposite the entrance of Mowry Slough). The Coyote Creek samples would be a good set of data to examine the Lowe and Thompson (1999) index. Other samples would be useful to determine if there are gradients in benthic communities which correlate with environmental gradients.

South Bay Benthic Data (1993-1995, 1997-1998): Single benthic samples were collected at 42-62 stations south of San Mateo Bridge from 1993-1995 and 1997-1998 during spring, summer, and fall. 6-11 stations were collected at or south of Dumbarton Bridge as follows: In March 1993, 8 stations were collected, in June 1993-September 1995 and

in March-June 1997, 11 stations were collected, in March-September 1998 6 stations were collected. The large bivalve species were removed and measured from all samples except during March 1997 and all of 1998. These data would be a good check to see if single station data is spatially representative and to see if benthic community gradients exist as proximity to wastewater plumes is decreased.

Charismatic Macrofauna - Some of the reviewers felt that additional information may be available on the dietary doses of copper and nickel that cause toxicity problems in birds and mammals, and that more information should be presented to rule out metal toxicity to birds and seals feeding on benthic macroinvertebrates and fish.

The final report will include avian and mammalian toxicity profiles for copper and nickel. Each toxicity profile will include a summary of available toxicity data and will include a rationale for selecting an appropriate toxicity benchmark that can be used to evaluate potential risks to charismatic macrofauna.

Individual Species Toxicity Tests - The reviewers agreed that individual species toxicity tests were appropriate for developing indicators of copper and nickel toxicity on the South Bay ecosystem. However, several comments were made concerning these tests. The assumption that "clean" laboratory water contains no complexing capacity was questioned since these measurements were not made, and since many of the tests were conducted before "clean" techniques were developed. The opposite issue, that without high complexing capacity designed into the test media, metal bioavailability could change during the tests due to exudation of ligands (increased complexing capacity) and metal uptake, was also discussed. Another concern was that the toxicity tests do not consider the effects of competition between metals or complexation with organics.

Dave Hansen expressed reservations about deleting non-resident species from the national Water Quality Criteria database, since these results could represent untested resident species, and since deleting species artificially lowers the Water Quality Criteria by reducing the number of GMAVs used to calculate the FAV. He also felt that the WER Cookbook rules for deleting species were not followed in the report. He also suggested that the Biotic Ligand Model should be discussed in the report, since it will soon be accepted as an alternative to the standard Water Quality Criteria approach.

The reviewers concerns that complexing capacity was not measured, that it could change during the experiments, and that competition between metals was not considered will be added to the discussion of uncertainties in the final report. The meaning behind the term "clean laboratory water" is not meant to imply that the "clean laboratory water" was either collected or prepared using the "clean" techniques that were developed only recently. The U.S. EPA toxicity testing protocols require that "clean laboratory water" be used for both control treatments and a dilution source (except in cases which use receiving water for dilution). By definition, "clean laboratory water" can be either artificially prepared (e.g., artificial seasalts) or naturally occurring waters, both of which must contain little or no metal complexing capacity (if metals are a toxicant of

concern). The terminology will be clarified and defined more concisely in the final report.

The procedure that was used to determine the "resident/surrogate" and "resident" categories was not based on the procedures recommended in the WER Cookbook (U.S. EPA 1994). The approach that was taken was to select as resident species those species or genera that have been reported in the literature to reside in the Lower South San Francisco Bay. The "surrogate" species that were included in the Impairment Assessment were those species that are commonly used/required as test species for dischargers into the San Francisco Bay in compliance with their NPDES permits. The final report will include an additional category that represents only those species that can be deleted according to the guidance provided in the WER Cookbook. The results of this additional analysis would most likely produce an SSO value that is greater than the value produced using the original data set of "resident and/or surrogate" species.

The phenomena of toxicant additivity, synergism, competition, antagonism, or chelation are very important in determining the causes of toxicity in a natural setting and cannot be ignored. The individual species toxicity tests, however, were designed to determine the toxicity of a specific toxicant (e.g., copper, nickel) and were not designed to determine the effects of multiple toxicants. This procedure reduces the number of test variables, makes data interpretation more definitive, and aids in determining the potential causes of toxicity in a complex system.

The Biotic Ligand Model was not discussed for several reasons. First, although it will soon be accepted as an alternative to the standard Water Quality Criteria approach, it was not an accepted approach at the time the Impairment Assessment was conducted. Second, this model deals with the effects of metal speciation on the accumulation of metals on fish gills and the resulting acute toxicity. Acute toxicity to fish was not a major issue of the Impairment Assessment. Finally, many other models are also available to predict metal accumulation and toxicity in aquatic organisms, but reviewing these models was not the focus of the Impairment Assessment.

AERAP - None of the reviewers were familiar with the AERAP methodology, so they suggested that it would be useful if someone else who knew the method could also review that section of the report. The addition of supporting references describing successful AERAP applications was also suggested. Ken Bruland commented that the description of the AERAP in the report is overselling its capabilities. The report suggests that the AERAP measures the toxic response of the community and ecosystem, when in fact it is limited to the species for which toxicity data are available. Dr. Bruland felt that a community ecological study would be necessary to first determine the key organisms and that toxicity tests would then have to be conducted on these organisms to provide the appropriate data for the AERAP analyses.

The description of the AERAP was taken from the original model documentation in the Water Environment Research Foundation project report. This description will be revised

in the final Impairment Assessment report to more accurately portray the capabilities of the AERAP as an assessment tool.

Dave Hansen had several concerns about using the AERAP methodology to develop SSOs. These are summarized below:

1) The calculated 95% level of protection was lower than expected based on the toxicity test values.

The AERAP is a logistic regression model that provides a best fit to the entire data set. Therefore, the 95% level of protection on the resulting logistic curve does not necessarily match the one or two toxicity test values at the extreme end of the curve. In this application, the AERAP value was lower than what was observed for the most sensitive species in the toxicity tests. This makes the result conservative as far as protecting the community, based on the existing toxicity data.

2) The confidence limits of the species sensitivity distribution were widest near the center of the distribution and narrowest at the two tails, which is the reverse of what occurs in most statistical analyses.

Dr. Jon Butcher, a co-author of the AERAP, is being consulted to provide a supplement that provides a more complete description of the statistical methods that are used in the AERAP. Dr. Butcher's supplement has not been completed. However, the final report will include this supplement and also address any implications the specific procedures have for the assessment.

Confidence limits for the AERAP model are developed using standard equations for the linear regression model. The confidence limits appear to collapse at the tails of the distribution because of the logistic transformation. That is, the regression is a logistic regression, conducted in logit space. On the logit scale, the confidence limits "spread" toward the tails of the regression, as expected intuitively. Back transformation to the probability scale of percent genera effected, as presented in the figures, results in an apparent collapsing of these confidence limits.

3) Species interactions are not considered, so the results may not be protective at elevated exposure concentrations.

We agree that species interactions are not included in the AERAP methodology. However, quantifying such effects are beyond the current capabilities of any methodology that attempts to extrapolate laboratory toxicity tests to make predictions in the field. In practice, we are generally trying to protect the more sensitive species, so we are operating at the lower end of the curve. By protecting the most sensitive species, we should also be protective of important species interactions.

4) ACRs from acutely sensitive species should not be applied to insensitive species, since ACRs typically increase as acute sensitivity decreases.

During the TRC meeting, Dave Hansen suggested that the AERAP analysis be conducted for acute toxicity with the ACR performed on the final Environmental Risk Concentration (ERC). This value will be used in a manner consistent with the EPA's Site-Specific WQC Guidelines, that require commercially and environmentally important species be protected. This procedure precludes the inclusion of plant and algal species since they are based on chronic endpoints (e.g., reduced growth versus mortality). The consequences of this approach will be presented and discussed in the final report.

5) Site-specific WERs from acutely sensitive species should not be applied to insensitive species, since WERs are typically highest for the most sensitive species and decrease as acute sensitivity decreases.

In the draft report, each species was adjusted using the City of San Jose site-specific WER value prior to AERAP analysis. An alternative that was discussed at the TRC meeting was to apply the City of San Jose site-specific WER to the ACR adjusted ERC. The consequences of this application will be presented and discussed in the final report. Any value obtained will be used in a manner consistent with the EPA's Site-Specific WQC Guidelines.

6) The use of different types of toxicity data from different types of organisms to develop a single statistical distribution may not be appropriate.

We agree that the combination of different types of toxicity data from different types of organisms presents potential conceptual problems. The mode of action of toxicity frequently differs systematically from one type of organism to the next. The interaction of different effects on different species could lead to a very complex chain of events that ultimately could have ecological consequences at a community level. The extrapolation of toxicity effects on individual species to a community or ecosystem level response is beyond current capabilities, including the AERAP.

However, the approach of combining acute toxicity data across different organisms is fully consistent with EPA's (1985) guidance, which states that development of a criterion for freshwater aquatic organisms should include results of acceptable acute tests with at least one species of freshwater animal in at least eight different families, specified to include fish, chordates, insects, and planktonic and benthic crustaceans. While the EPA FAV is derived from a selected subset of GMAVs, no provision is made for the effects of combining different phyla. For instance, the four most sensitive genera for nickel acute toxicity include both crustaceans and fish.

7) Deletion of data for non-resident species should follow the WER Cookbook.

This issue was addressed above.

8) The assumptions of the statistical model should be described.

Dr. Butcher's supplement to the final report will describe the statistical assumptions of the logistic regression model.

Site-Specific Studies - Dave Hansen commented that although the site-specific studies conducted by the City of San Jose appeared to be very well done and were used appropriately in the report, he had some reservations about the deletion of non-resident species from the national Water Quality Criteria database, and about the site-specific study for nickel.

The site-specific studies for nickel performed by the City of San Jose used the methodologies described in the WER Cookbook (U.S. EPA, 1994).

Phytoplankton - The reviewers agreed that the discussion of phytoplankton uptake and toxicity was current and that additional phytoplankton toxicity studies should be conducted, since phytoplankton appear to be the most sensitive organisms to copper toxicity in the bay. Several reviewers felt that phytoplankton should have been selected as a primary indicator because of their high sensitivity and their importance at the base of the food chain. Jonathan Phinney suggested that lipophilic organic metal complexes should also be discussed, since these complexes are very bioavailable and since industrial sources of the ligands may exist in the South Bay. Jim Kuwabara suggested that possible antagonistic effects of elevated silica on copper toxicity to diatoms should also be addressed.

Discussions of lipophilic organic metal complexes and silica/copper antagonism will be added to the report. In addition, recent evidence has emerged since the review meeting that cyanobacteria, believed to be the most sensitive phytoplankton to copper toxicity, do occur in the Lower South Bay. These phytoplankton were previously thought to be absent, possibly due to copper toxicity. Recent studies in San Francisco Bay (including the Lower South Bay) have found the cyanobacterium (Synechococcus) present in all samples from all cruises between April and August, 1998 (Jim Cloern, USGS, personal communication, 1999). Although the abundances were not high, the researchers indicated that they believed this was a result of the particular combination of temperature and nutrients in the Bay. In addition, Brian Palenik (UCSD) has found cyanobacteria to be present at concentrations up to 50,000 cells/ml in the South Bay in July 1999, similar to levels seen in Southern California coastal waters. However, cyanobacteria concentrations were near detection limits during the January and April 1999 sampling event in the South Bay, while concentrations of 1,000 to 6,000 cells/ml were measured in the North Bay during these periods. Enrichments from the South Bay samples showed that at least three types of cyanobacteria were present, two likely related to Synechococcus and one resembling Synechocystis. These studies indicate that cyanobacteria are present in the Lower South Bay during the seasonal period when copper concentrations are greatest. These new studies will be examined by the Impairment Assessment team, along with the other existing phytoplankton data, and recommendations will be made in the final report regarding possible special studies that are based on their findings.

4) **Do the findings and recommendations in the report follow logically from the data and scientific information presented?**

The reviewers did not respond to this question since they felt it was too broad. They wanted more specifics on which particular findings and recommendations needed to be addressed. However, they felt that their responses to the other questions and their comments on the report would probably address all of the important issues implied by this question.

5) **Based on available evidence, can the null hypothesis that Cu and Ni impairment in the lower south San Francisco Bay exists be rejected?**

The reviewers agreed that this null hypothesis cannot be rejected without additional information on the toxicity of copper to sensitive phytoplankton (cyanobacteria) in the South Bay. Such studies were also recommended in the report. They also agreed that although the existing data suggests that invertebrates and fish are not impaired by the metals, the issue of potential sediment toxicity needs to be evaluated more thoroughly.

As mentioned above, recent evidence indicates that sensitive species of cyanobacteria (Synechococcus) do occur in the Lower South Bay. These new findings will be examined by the Impairment Assessment team who will then look at all of the existing phytoplankton data and make recommendations in the final report regarding possible special studies that are based on their findings.

Sediment toxicity and benthic macroinvertebrates will be addressed more thoroughly in the final report, as described above in Question 3 (SEM/AVS and Benthic Macroinvertebrate sections).

6) **The report describes a technical basis for establishing site-specific water quality objectives for copper and nickel at several different levels within an overall range. In your opinion, which specific concentration value for copper is best supported by the technical evidence and why? Please answer the same question for nickel.**

The reviewers did not select specific SSOs for copper and nickel since they were not able to conduct thorough reviews of all of the data and calculations used to derive them. The reviewers generally felt comfortable with the values presented in the report, with the reservation that copper toxicity to phytoplankton needed further study. Dr. Jonathan Phinney indicated that he felt that the range was protective of aquatic life in the South Bay, but that the phytoplankton issue required additional study before any definitive statement could be made.

Dave Hansen outlined the approach he prefers for deriving SSO values. This was: (1) follow national WQC guidelines, (2) use all available data that meet the guideline standards, (3) do not delete nonresident species (but use WER Cookbook rules if you do), (4) use WERs to adjust the resulting values for site-specific water quality conditions, (5)

use the different WER values at different locations in the South Bay to calculate different SSOs at each location.

In general, the guidelines that Dr. Hansen preferred were utilized to the extent that was possible with the AERAP model. For the "National" and "National/No Plants" categories, all of the available data that were currently in the U.S. EPA data set were used, with no deletions. In all cases, the site-specific WER values obtained by the City of San Jose (1998) were used to adjust the resulting values for site-specific water quality conditions. As mentioned above, the "resident and/or surrogate" categories that were used included test organisms that were actually known to be residents of the Lower South Bay or were commonly used surrogate test species in South Bay discharger NPDES permits. The final report will include an additional category that meets the WER Cookbook rules for species deletion.

While the San Jose WER studies indicated that there were several potentially acceptable WER values for the study area, the recommendation to set separate SSOs for various parts of the Lower South Bay is a stakeholder decision. This approach would also introduce issues concerning the boundaries of the areas where each SSO was applicable.

7) Have the most important uncertainties been identified?

The reviewers felt that most of the important uncertainties had been identified, but suggested a few additional uncertainties that should be mentioned in the report. These are listed below.

Bioavailability of particulate metals to invertebrates and fish is poorly understood, but should be addressed in the report.

Limited data are available on the chemical speciation and complexation of copper and nickel in the South Bay to assess phytoplankton toxicity.

The characteristics of the control waters used to calculate WERs are often not well known and need to be carefully evaluated.

The discussion of uncertainties associated with toxicity tests should also address uncertainties associated with the use of toxicity data in making extrapolations to predict site-specific effects in the field. This includes uncertainties in the use of toxicity data to derive water quality criteria, and in applying the AERAP methodology.

Discussion of the uncertainties associated with items 1, 3, and 4 above will be added to the report. Item 2 was discussed and studies were recommended to reduce this uncertainty.

In addition to uncertainties concerning the Impairment Assessment, the reviewers also identified some additional uncertainties concerning copper and nickel cycling in the South Bay. These include limited knowledge of the effects of benthic invertebrates on

copper and nickel remineralization from suspended particles during filtration and digestion, benthic bioturbation/irrigation effects on sediment release fluxes (biologically enhanced advection), and the lack of knowledge on adsorption/desorption kinetics and the release of the metals from resuspended sediments.

The latter uncertainty was addressed in the Conceptual Model Report, and additional work is currently under way to address this issue further. The effects of benthos on copper and nickel cycling were also addressed in the Conceptual Model Report, but the discussion will be expanded to identify this as a major source of uncertainty. This updated information will also be included in the final Impairment Assessment Report.

8) To what extent will the proposed special studies reduce or eliminate the uncertainties?

The reviewers agreed that there will probably always be some uncertainties regarding metals toxicity in the South Bay, but that the proposed studies would reduce the current uncertainties in copper and nickel toxicity. If the results of these studies indicate that phytoplankton toxicity is not occurring because of competition with other metals (Mn, Zn, Fe) and/or complexation with organics, and if it can be established that copper and nickel are not sources of sediment toxicity, then the risk of impairment would be low.

Several suggestions were given concerning the recommended studies.

1. The phytoplankton toxicity tests should use the most sensitive species (cyanobacteria) and should include tests using South Bay water.

2. Water from several sites in the South Bay should be tested during both the dry and wet seasons.

3. Phytoplankton toxicity should be related to measurements of stress proteins rather than just total metal concentrations in cells, since phytoplankton can adapt to high metal concentrations by producing phytochelatins, which sequester the metals in the cell in nontoxic forms.

4. Speciation studies should be conducted for Cu, Zn, Mn, and possibly Fe if phytoplankton toxicity occurs to determine if metals are causing the toxicity.

5. The speciation and complexation studies may require modeling, since some of the analytical techniques required to measure the different organic metal complexes are still under development.

6. The study designs should be reviewed to ensure that they will provide the necessary information to resolve the phytoplankton toxicity issue.

9) In the report, four Environmental Risk Concentration Values (ERCs) are presented, which are calculated using the AERAP model from four different

toxicity databases. The ERC based on the National database is very similar to (but slightly higher than) the ERC based on the National/No plants, even though laboratory toxicity tests show that some species of algae, including *T. pseudonana*, are among the most sensitive organisms to copper. In developing criteria, EPA calculates a final acute value (FAV) from the four most sensitive species, divides by an acute-to-chronic ratio to arrive at a CCC. In the Report, the ERCs are treated as equivalent to a CCC in calculating possible site-specific criteria (i.e. they are multiplied by the WER). In developing a criterion that is protective of plants, is it more appropriate to use the AERAP or FAV method?

The reviewers were less familiar with the AERAP method than the FAV method, but felt that both methods could be protective of plants if the appropriate data were used. The AERAP used all available data (including plants and algae) whereas the FAV method does not include any plant or algae data. The plant and algae data used by the AERAP originated from the U.S. EPA Water Quality Criteria database that includes a range of plant and algal sensitivities. The major concern was that data exist that suggest different sensitivities between different types of phytoplankton (cyanobacteria, coccolithophores, dinoflagellates, and diatoms), and that the most sensitive species (cyanobacteria) are not adequately represented in the database. Another concern was that the toxicity database does not consider the effects of competition between metals or complexation with organics.

Both concerns would be resolved by the recommended phytoplankton toxicity studies. The WER procedure attempts to account for the effects of the second concern.

10) Please evaluate (1) the level of conservatism of each of the key technical assumptions leading to the SSO (e.g., resident/surrogate species selection, AERAP % species protection, ACR value, 2 vs 3 station WER (i.e. geographic extent) and (2) the cumulative impact of these individual assumptions on the conservatism of the resultant SSO.

The reviewers generally agreed that the technical assumptions were conservative and that if the lower metal concentration is selected for each of these assumptions, the cumulative impact would be low and the resultant SSO would be conservative. Dave Hansen expressed some reservations about deleting data for species that are not in the South Bay since they may be relevant to other species in the bay that have not been tested. He also suggested using the different WER values to develop different SSOs at the corresponding locations in the bay. He also expressed some concerns about the AERAP values selected, which were either outside the range of the database or did not seem to fit the appropriate percentage within the database.

The rationale for the existing "resident and/or surrogate" categories has been explained above, as well as the intention to include an additional category that includes species that meet the WER Cookbook's guidelines for species deletion.

> *The use of the two-station WER value versus the three-station WER value was done to provide an additional measure of conservatism. This is also consistent with the procedure used in the City of San Jose Site-Specific Study (1998). The rationale was to provide the greatest level of protection for the broadest area within lower South San Francisco Bay. The technical project team will need further direction from the TWG and the Regional Water Quality Control Board before applying different WER values for different locations in the impairment assessment analysis.*

> The AERAP is a logistic regression model that has the ability to predict toxicity for species other than those included in the model's database. The purpose is to predict the 5% toxicity level for all the species that could be represented within the biological community of lower South San Francisco Bay. The calculation of the 5% level is not made solely on the species included in the database. That is, if there are 20 species, the most sensitive species does not necessarily represent the community 5% Environmental Risk Concentration. The model fit may predict a concentration that is lower than the most sensitive species in the database. This will be discussed further in the final report.

2.3 Additional Comments by the TRC

In addition to their responses to the above questions, the TRC also had several additional comments concerning the report. These are summarized below. The Tetra Tech impairment assessment team responses to the TRC comments are presented in italics following each of the reviewer comments.

Water Quality Modeling for Averaging Period and Return Frequency Issues - Dave Hansen commented that averaging period and return frequency were not discussed along with the recommended SSOs, and that water quality modeling was necessary to establish these relationships.

Water quality modeling was not deemed necessary due to the extensive monitoring data collected by the City of San Jose and the RMP. Although daily variations in copper and nickel concentrations were not measured, these differences are expected to be small considering the consistent seasonal and spatial trends in the data and the fact that different portions of the tidal cycle are sampled on different dates. The monitoring data show that copper concentrations have consistently been below the recommended SSOs during the last 5 years, and that nickel concentrations are also generally below the recommended SSOs for nickel.

Reason Why AERAP Was Not Used for Nickel – Dave Hansen asked why the AERAP analysis was not conducted for nickel.

The AERAP analysis was not used as an assessment tool because its addition would not have added any additional value to the results obtained by the City of San Jose during their "Recalculation of the National Marine Water Quality Criterion and Development of a Site-Specific Nickel Criterion" study. This study used the procedures described in the WER Cookbook to update the National data set and recalculate a new national water quality criterion for nickel that was based on site-specific species composition. Since this procedure had already

been used by the City to calculate several potential site-specific water quality objectives for the South Bay, the Impairment Assessment team did not believe that it was necessary to repeat the process using the AERAP procedure.

Linkage Between Impairment Assessment and Other Reports - Jim Kuwabara suggested that it would be useful to discuss the linkage and integration between the Impairment Assessment and the other reports (Conceptual Model, Source Characterization, Hydrodynamic Modeling), since the physical, biogeochemical, and biological processes that determine the distributions of Cu and Ni in the South Bay will be important in developing site-specific objectives, TMDLs, and waste allocations.

This discussion will be added to the introduction of the final report.

Questions and Comments About Previous Studies - The reviewers had several questions and comments about the results of some of the earlier studies described in the report. These ranged from questions about high variability or data inconsistencies in some of the 1991-92 toxicity and WER studies to comments that some of the calculation procedures used in these studies are no longer acceptable.

These studies were presented in the report only to provide historical perspective. They were not used to develop the site-specific objectives in the Impairment Assessment Report since more recent studies were available. The calculation procedures used by the earlier studies were acceptable at the time those studies were conducted. The data presented in Tables 4-7 through 4-9 were taken directly as presented in the cited reports.

Comments about Indicator Species and Resident Species - Some reviewers commented about the description of indicator species and resident species procedures in the report. Dave Hansen felt that the complexity of the methodology was not captured.

These descriptions are those provided in "Guidelines for Deriving Numerical Aquatic Site-Specific Water Quality Criteria by Modifying National Criteria" (Carlson et al., 1984).

Ken Bruland commented that species from laboratory culture collections can be more metal tolerant than species in the field. Jim Kuwabara commented that metal tolerance can be developed rapidly in phytoplankton, so that species collected from areas in the bay with elevated metals may be more tolerant than species from uncontaminated areas.

These issues concerning metal tolerance will be added to the discussion of uncertainties in the final report.

Significant Figures - The reviewers commented that too many significant figures were reported for some of the metal concentrations that were based on calculations.

This was done since the WER Cookbook specifies that 4 significant figures must be reported to prevent round-off errors from accumulating in the analyses. The WER Cookbook (U.S. EPA, 1994) states that,

"To prevent round-off error in subsequent calculations, at least four significant digits ***must*** *be retained in all endpoints, WERs, and FWERs. This requirement is not based on mathematics or statistics and does not reflect the precision of the value; its purpose is to minimize concern about the effects of rounding off on a site-specific criterion. All of these numbers are intermediate values in the calculation of permit limits and should not be rounded off as if they were values of ultimate concern."*

Editorial Changes - Several minor editorial changes were suggested by the reviewers. These ranged from minor changes in wording to additional clarification of a few statements to missing references (e.g., "Gold Book").

These corrections will be made to the final report.

Dave Hansen commented that the glossary of terms does not follow the context of WQC derivation and that Stephan et al. (1985) had nothing to do with site-specific WQC.

The descriptions of the glossary terms were paraphrased from Stephan et al. (1985). The final report will contain the entire description of each term as presented in that document. The citation of Stephan et al. (1985) with reference to site-specific WQC was an error. The correct citation is Carlson et al. (1984), and will be corrected in the final report.

3. Preparation of the Final Impairment Assessment Report

The draft Impairment Assessment Report will be revised based on the reviewers' comments to incorporate the changes as described above. The reviewer comments, as well as this summary report, will be included as an appendix to the final report. The introduction to the Impairment Assessment Report will be modified to acknowledge the reviewer's contributions and to direct the reader to the reviewer's comments.

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TMDL Work Group Memorandum

FROM: Tom Grieb, Tetra Tech

TO: Ken Bruland, Dave Hansen, Jim Kuwabara, Jonathan Phinney

DATE: September 7, 1999

SUBJECT: Review of Impairment Assessment Report for Copper and Nickel in Lower South San Francisco Bay

This package contains the following documents:

1. **Draft Final Conceptual Model Report**. This document summarizes the existing knowledge of the behavior of copper and nickel in Lower South San Francisco Bay. This is the version of the document that was reviewed by the previous Technical Review Committee. Several changes will be made to the final version of the document. Major changes will include the preparation of separate diagrams and estimates of concentrations and fluxes for the wet and dry seasons.

2. **Report to the TMDL Work Group on the Technical Review Committee Review of the Conceptual Model Report for Copper and Nickel in Lower South San Francisco Bay.** This document summarizes the technical review process for the Conceptual Model Report. This document includes a description of the review meeting and the comments of the previous reviewers.

3. **Task 9 – Technical Review. Technical Review Committee Procedures Document.** A description of the technical review process was prepared early in the TMDL project. This document describes the overall approach that was envisioned.

Another document describing the City of San Jose's Water Effects Ratio Study is being sent directly from the City. These documents are provided as background materials. We are not trying to inundate you with data and information. Please contact me if you have any questions about the information that is being provided.

Thank you

Questions Submitted to the TRC

1.   General Questions

- Is the method or approach for evaluating impairment in the Report reasonable?

- Are all of the relevant data included or summarized adequately in the Report?

- Does the discussion of indicators reflect the current state of scientific knowledge on the relevant topics?

- Do the findings and recommendations in the Report follow logically from the data and scientific information presented?

- Based on available evidence, can the null hypothesis that Cu and Ni impairment in the Lower South San Francisco Bay exists be rejected?

- The Report describes a technical basis for establishing site-specific water quality objectives for copper and nickel at several different levels within an overall range. In your opinion, which specific concentration value for copper is best supported by the technical evidence and why? Please answer the same question for nickel.

2.   Questions Related to Uncertainties and Special Studies

- Have the most important uncertainties been identified?

- To what extent will the proposed special studies reduce or eliminate these uncertainties?

3.   Specific Questions

- In developing a criterion for copper that is protective of plants, is it more appropriate to use the AERAP or FAV method?

- Please evaluate 1) the level of conservatism of each of the key technical assumptions leading up to the SSO, and 2) the cumulative impact of these individual assumptions on the conservatism of the resultant recommended SSO.

Calculation of TMDLs for Copper and Nickel in South San Francisco Bay

Tom Grieb, Tetra Tech, Inc.
Adam Olivieri, EOA, Inc.

The emergence of the TMDL process as an important planning and regulatory decision-making tool is a recent development in national, regional, and local efforts to achieve continued improvement in the quality of the nation's surface waters. The TMDL, or total maximum daily load, establishes the allowable loadings of a pollutant that a water body can receive without violating applicable water quality standards or harming beneficial uses. Although identified in Section 303(d) of the federal Clean Water Act (CWA) over 20 years ago, it is only since 1996 that the TMDL has become an important process for developing state water quality standards.

The development of TMDLs for copper and nickel is required because South San Francisco Bay (South Bay) has been designated an impaired water body under Section 303(d) of the CWA. Although this is a requirement, there is also optimism that these TMDLs will provide a unique opportunity to address the many complex issues associated with setting water quality standards for the South Bay. Stefan Lorenzato, the TMDL coordinator at the State Water Resources Control Board, notes that the collaborative approach that is being taken to prepare these TMDLs is likely to be more successful than the programmatic approach that has traditionally been used by state and local regulatory agencies.

These copper and nickel TMDLs are noteworthy for several reasons. Foremost among them is the fact that they are being independently funded by the City of San Jose. David Tucker and Dan Bruinsma, the City of San Jose's co-project managers, note that "*This is one of the most comprehensive, chemical-specific, environmental assessments ever conducted in San Francisco Bay; a total of $3.5 million has been allocated by the City for this 4-year effort.*" The copper and nickel TMDLs are also being integrated into the ongoing Santa Clara Basin Watershed Management Initiative (WMI), and a major emphasis is being placed on establishing and maintaining public and industry involvement. One indication of the collaborative aspect of this effort, referred to above by Stefan Lorenzato, is the formation of a TMDL Work Group (TWG). The TWG is made up of stakeholders from wastewater and stormwater dischargers, environmental groups, industry, regulatory agencies, and other involved citizens, and it has been formed as part of the WMI's Bay Modeling and Monitoring Subgroup. The charter of this group is to guide the TMDL process and to develop new and preferred ways to make the process understandable and equitable. A Technical Review Committee (TRC) has also been formed to review the technical products of the TMDL effort. The TRC is made up of nationally recognized technical experts in such areas as the behavior of metals in aquatic systems, hydrodynamics, estuarine modeling, ecological effects of trace metals, sediment transport processes, and atmospheric modeling.

The focus of the copper and nickel TMDL efforts during the first year of activity has been in the following five primary areas of investigation:

Data Collection and Analysis. One of the first efforts has been to create an extensive database that is available to both technical and stakeholder personnel involved in the project. The database is unique in that it brings together different types and large volumes of information (over 1.5 million records have been entered so far) focused on the specific issues of TMDL development for copper and nickel in the Lower South San Francisco Bay. Many investigators in the area have contributed to the development of a database that consists of water quality data, sediment quality data, sediment core data, point and nonpoint source loading data, basemap information, bathymetric data, hydrodynamic data, suspended solids data, air quality data, and photographic/satellite imagery.

Additional data will continually be entered, as they become available during the project. To facilitate use and understanding of the data, the database has been created in a Geographic Information System (GIS).

Conceptual Model Development. A conceptual model that depicts the current understanding of the processes that influence copper and nickel cycling in Lower South San Francisco Bay and adjacent Bay waters was recently produced. To communicate the information that has been developed on loadings, sediment transport and copper and nickel cycling, the conceptual model makes extensive use of graphics. The objective of this effort was to develop a tool for effectively communicating the existing information to a wide audience of interested stakeholders. Diagrams such as the one shown in the accompanying figure can be used to facilitate the discussions of upcoming TMDL issues such as source characterization, beneficial-use impairment, simulation model development, and the design of special studies. The conceptual model was the topic of one of the poster sessions at the recent State of the Estuary Conference.

Source Characterization. The major sources of copper and nickel that enter the South Bay are being quantified. The loadings have been divided into four major source categories: wastewater discharges, tributary loads, atmospheric deposition to the surface water, and sediment exchange with the water column within the Bay. This effort is the first step in identifying the major contributors of copper and nickel loading so that appropriate control measures can be developed if necessary. It is also the purpose of this work to identify limitations and uncertainties in the existing loading data so that additional efforts to improve these estimates can be focused in the appropriate areas.

Assessment of Beneficial Use Impairment. In January of this year, over 50 individuals from local regulatory agencies, municipal dischargers, stormwater management groups, environmental groups, and other South Bay stakeholder groups participated in an impairment assessment workshop held at the San Francisco Bay Regional Water Quality Control Board. Information was presented on progress made in developing indicators for assessing impairment to beneficial uses. The results of the workshop were also presented at the recent State of the Estuary Conference. Later this spring, an Impairment Assessment Report will be completed. The purpose of the impairment assessment is to determine if and when and how the beneficial uses of the South Bay are adversely affected by copper and nickel, and what concentrations cause these

problems. The results of this assessment will determine the course of all further activities associated with these TMDLs.

Simulation Model Development. The first of several technical reports that will be produced in the evaluation of existing two- and three-dimensional numerical simulation models was completed in December 1998. This document identifies models that could be used in the calculation of TMDLs for copper and nickel in South San Francisco Bay. This evaluation process is important because numerical models will be the primary tool used to evaluate the responses of the South Bay to copper and nickel loading. This initial report identifies the model components that are necessary to simulate and predict the transport and fate of copper and nickel in South San Francisco Bay. Twenty potentially applicable models were identified and classified according to type and functionality, and a subset of 10 models was recommended for further evaluation.

Comments on the TMDL Process
Numerous individuals in the copper and nickel TWG have already made significant time commitments to this process. Tom Mumley of the California Regional Water Quality Control Board and the TWG's co-chairman suggests that "*This is because many people recognize that the up-front involvement of the stakeholders and the level of funding available offers a unique opportunity to achieve resolution of issues that are acknowledged to be both politically contentious and technically complex.*" Rainer Hoenicke, the other TWG co-chairman and the program manager for the Regional Monitoring Program for Trace Substances, also points out that "*The information synthesis effort that is part of the problem characterization is particularly relevant, because for most of the stakeholders, this is an invaluable opportunity to become educated about the complex issues surrounding these two metals.*" Also, as the program manager for the Regional Monitoring Program for Trace Substances, he is personally excited about the TMDL effort because it demonstrates that the monitoring activities conducted in the estuary will have an impact on environmental decision-making. He is also hopeful that the conceptual model and the other problem definition efforts of the TMDL will help to focus future data collection efforts. Michael Stanley-Jones of the Silicon Valley Toxics Coalition and CLEAN South Bay's environmental coordinator for the Copper-Nickel TMDL has expressed optimism that the tools that are being developed for these TMDLs will provide a strong technical foundation for future TMDL efforts in the San Francisco Bay/Estuary.

Written Comments from the Technical Review Committee Members

Comments from Ken Bruland

From: bruland@cats.ucsc.edu
Sent: Wednesday, September 22, 1999 1:46 PM
To: Tom.Grieb@tetratech.com
Cc: kuwabara@usgs.gov; bruland@cats.ucsc.edu; JPhinney@dccmc.org;
Dhansen334@aol.com; tem@rb2.swrcb.ca.gov
Subject: Review (TMDL - Resource Impairment Report)

Technical Review of Impairment Assessment Report - Ken Bruland (UCSC)

Pg. 2-5 Aquatic Ecological Risk Assessment Protocol (AERAP):

The description of this protocol needs to be more carefully worded. It is a model based upon the toxicity data base that exists for individual organisms for which there is data. They make it sound like it is "evaluating the ecologically relevant measure of community status." They state that "this indicator provides a measure of the assemblage of species necessary to support a dynamic and productive trophic structure." This language is overselling what an AERAP provides. If a careful community ecological study was performed and it was ensured that the key organisms which support the dynamic and productive trophic structure were chosen for toxicity studies to be performed, then it might achieve this goal. As it stands, the AERAP indicator provides a measure of the assemblage of species for which individual toxicity data exists - not necessarily the key organisms supporting the community.

Pg. 2-6 Plankton

The South Bay has a phytoplankton community dominated by diatoms. Diatoms are a key indicator because of their position at the base of the food chain. Yet plankton is not recommended as a primary indicator in the assessment.

Pg 2-6 Benthic Macroinvertebrates

Reportedly, 60% of the sediments in the South Bay are toxic. We can not rule out that Cu and Ni may contribute to this toxicity. This is an area where there is a fair amount of literature and studies that appear not to have been evaluated (e.g., the work of Sam Luoma at USGS). I don't think that the statements in this section have been adequately justified.

Pg. 2-7 SEM/AVS

The statement that "the method has not been used in any known monitoring program in Lower South San Francisco Bay" may not be true. Reportedly there have been studies carried out (check with Sam Luoma (USGS) or Byeong Lee). In addition, there have been pore water studies performed (check with scientists such as Russ Flegal (UCSC) and Will Berelson (USC)/ Kenneth Coale (MLML)).

Pg. 2-9 Birds

I was not completely convinced that birds that feed upon benthic macroinvertebrates with elevated levels of Cu and Ni should not be considered. It seems like a fairly straightforward question to address.

Pg. 3-2 Water Quality Data

It would help me assess the quality of the data if the name of the research lab that carried out the measurements was mentioned. For example, instead of just the Regional Monitoring Program data, credit should also go to the lab that made the measurements. As long as the RMP has Russ Flegal's lab (UCSC) make these measurements, I am confident of the data quality. If the RMP switched to another lab to make these analyses, then I would have to reevaluate the quality of the data, and from my perspective, it may not be "quality data". There is a tremendous data base available, but one still has to be extremely careful. The Regional Monitoring Program deserves to be congratulated on the data quality - as a result of contracting a quality lab to make the measurements. The City of San Jose lab (SJSB) also appears to be providing quality data. I am unable to evaluate the earlier data sets from the South Bay discharge Authority (SBDA) without knowing what lab made these measurements.

Pg. 3-6

This section on desorption and adsorption rates makes it sound as if these values are known with a high degree of confidence. It states things as apparent facts. For example, "Desorption rates into the dissolved phase exceed adsorption rates back to the adsorbed phase, so desorption during resuspension is a major source of dissolved metals to the water column." This may be true, but it is not based upon any data from the South Bay. This was derived from a conceptual model where the rate constants were unknown and only very crudely estimated from studies carried out in Rhode Island and from basin sediments off Southern California (see the Wood et al paper).

Pg. 3-11

I'm not sure what, if any, conclusions can be drawn from the 1992 studies by S.R. Hansen & Associates. The ambient site-water controls exhibited toxicity. I'm not convinced these studies were carried out cleanly enough.

Pg. 4-5

I'm not convinced we should be putting much weight on the WER's from the early studies by S.R. Hansen & Associates. The data I trust are the more recent values from the City of San Jose where they determine consistent WER's with a low variability.

Pg. 4-5, 4-6

Far too many significant figures are being reported here for the metal concentrations. When final metal concentrations are mentioned, there should only be two significant figures. A value of 8.3 ug/L should be used, rather than 8.293 ug/L.

Pg. 4-7 Selecting resident and surrogate species

It can not be assumed that "clean" laboratory water contains little or no metal complexing capacity. These measurements were not made.

Pg. 4-16

What is the "Gold Book". I could not find the reference.

Pg. 4-23

Blue-green algae are not necessarily nitrogen fixing. The most common photosynthetic cyanobacteria in marine waters are not nitrogen fixing.

Pg. 4-55

Conclusion #2 and #4 are not valid. They stated that "the amount of bioavailable copper decreases in the Lower South Bay on a north to south basis." This is not necessarily true. What they can say is that relative to the same addition of dissolved Cu to the water, that the added Cu does not have as much of a toxic effect .." What has been documented is that for a given addition of Cu, that the Cu is not as toxic in the lower South Bay.

General Comments:

Areas of great uncertainty with respect to concentrations of dissolved Cu and Ni and their sources to the South Bay water column have to do with: 1) There is a lack of knowledge of the exchange kinetics between Cu and Ni sorbed or associated with surfaces of suspended particles or resuspended sediments, and soluble Cu and Ni species. This is particularly important with respect to estimating how large the source of Cu and Ni is from desorption or release from resuspended contaminated sediments. According to crude estimates, this is by far the largest source (and sink) of dissolved Cu and Ni to (and from) the South Bay water column. 2) There is

also very little knowledge of the role of benthic macroinvetebrates such as the asian clam in filtering suspended particles and remineralizing Cu and Ni as part of their digestion process.

These benthic macroinvetebrates are the main grazers of the phytoplankton and detritus in the South Bay. These comments are perhaps directed more towards the conceptual model of Cu and Ni in the South Bay. But best estimates of these rates are necessary to better evaluate the sources of dissolved Cu and Ni in the South Bay.

An area of uncertainty with respect to the toxicity of Cu and Ni to plankton is with respect to their chemical speciation and the degree to which these two metals exist as relatively inert organic chelates. This is particularly important with respect to the toxicity to plankton, where it is the free metal ion concentration that is the critical factor. There is only very limited data available in the South Bay to evaluate. We need a better link between toxicology and water chemistry.

The use of WER's can be useful. However, they need to be done with caution and the toxicity of the "control" or "clean" water needs to be carefully evaluated. The calculation of WER's then needs to be relative to the "control" or "clean" water. Ideally, we would like to understand the chemical reasons underpinning the use of WER's, rather than just treating it as a numerical parameter to use in comparing different sites.

General Questions:

1. Is the method or approach for evaluating impairment in the Report reasonable?

It is appropriate for the level of current understanding.

2. Are all the relevant data included or summarized adequately in the Report?

In most cases, yes. In a few cases, no. In particular I don't think they did justice to the potential sediment toxicity issues. There are relevant studies that were overlooked.

3. Does the discussion of indicators reflect the current state of scientific knowledge on the relevant topics?

Yes, with perhaps some question on the use of AERAP calculations.

4. Do the findings and recommendations in the Report follow logically from the data and scientific information presented.

Yes.

5. Based on available scientific evidence, can the null hypothesis that Cu and Ni impairment in the Lower South San Francisco Bay exists be rejected?

No, in particular for phytoplankton.

6. This question is a risk management decision.

Comments from David Hansen

TMDL Review
(September 13, 1999)

David J. Hansen

A meeting was held on September 13, 1999 to discuss and review the August 30, 1999 draft of the Impairment Assessment Report prepared by Tetra Tech Inc. The meeting began with a brief presentation to the review committee of the content of the report. The review committee consisted of Ken Bruland, Jonathan Phinney, Jim Kuwabara and David Hansen. The committee responded verbally to the content of the report and presentation as the presentation progressed. Following the presentation the committee answered six general questions prepared by the TMDL Work Group Subcommittee and an additional two questions related to uncertainties and special studies. The following are my individual responses to the questions and some additional comments on the Impairment Assessment Report:

General Comment: The Tetra Tech report was generally well written and scientifically sound. There is always great difficulty in presenting scientifically complex data sets with the goal of making a regulatory decision. I applaud the job done by Tetra Tech in preparing this report and especially the TMDL Work Group for their cooperative efforts in this complex undertaking. The comparisons in the report of ambient concentrations of dissolved metals with various estimates of effect concentrations based on the indicators were particularly insightful. The effect concentrations based on acute and chronic toxicity tests with embryos, larvae, juvenile and adult fishes and macroinvertebrates were greater than measured dissolved concentrations of copper or nickel, indicating that impacts to these life stages and taxa are unlikely. Concern was raised in the report, and by the Review Committee at the meeting, that certain phytoplankters may be sensitive at concentrations less than those measured. In addition, existing scientific methodologies need to be used to rule out metals as a cause of the pervasive sediment toxicity. A water quality model needs to be used to compare SSOs to concentrations in water needed with the averaging periods and return frequencies considered.

General Questions:

Question 1: Is the method or approach for evaluating impairment in the report reasonable?

Answer: The approach of developing "indicators" based on dissolved concentrations of copper and nickel and comparing them to ambient concentrations of dissolved metal is appropriate.

Question 2: Are all of the relevant data included or summarized adequately in the report?

Answer: I was encouraged to see that all of the aquatic toxicity data that I was aware of was summarized in Appendix C and used in indicator development. This included the historical data from the WQC documents and the newer data from the many site-specific studies. I was pleased

to see that the data from the study conducted by the city of San Jose was highlighted by Tetra Tech in the presentation of copper and nickel toxicity in "laboratory water, toxicity of copper in site water, metals concentrations in south San Francisco Bay, and in the site-specific studies themselves.   I am confident that there are some data out there were missed that is unknown to the authors of the report or me. The good news is that the WQC guidelines methodologies are robust and rarely does the addition of new data change the recommended final indicator concentration by a significant amount.

I was impressed with the approaches used to present the data. Figures 3-1 to 3-4 were exceptions. They are examples of computer based imaging at its best and worst. The vertical bars that were meant to demonstrate relative concentrations, were not useful as no scale was provided. If these figures were meant to indicate relative concentrations they were not useful. This is because the concentration scale was absent on any one figure, and there was no indication that the scale was the same for the four figures.

Question 3: Does the discussion of indicators reflect the current state of scientific knowledge on the relevant topics?

Answer: Significant improvement is needed in the discussion of indicators.

Community Analysis: Tetra Tech was correct in not including community analysis as a technique to assess the implications of copper and nickel in south San Francisco Bay. This is because this analysis can not be causally linked to copper, nickel or any other specific stressor. However, a comprehensive discussion of what is known about the health of the south bay ecosystem relative to similar systems would be invaluable. This discussion might prove to be the pivotal issue if the results of special toxicity assessments with possibly cyanobacteria, cocolithophores, dinoflagellates, and diatoms continue to indicate that some phytoplankters are more sensitive to copper than invertebrates and fishes.

WQC are not intended to protect all invertebrates, fish or primary producers all of the time. They are intended to protect most of the aquatic organisms most of the time. If special studies with phytoplankton conducted with key bay species indicate that effects might be occur, the WQC guidelines do not call for lowering the criteria concentration to protect all phytoplankton. The issue then becomes the selection of the appropriate level of protection required. One could use an approach like that used to derive the FAV once a level of protection is selected and keeping in mind recovery rates for phytoplankton. Alternatively, community assessments might be used to examine ecosystem health in south San Francisco Bay. It is for this reason, and not as an indicator of copper or nickels effects, that a more comprehensive examination of the results of community assessments would be valuable. For example, the effects of copper on a freshwater ecosystem has been tested in a study conducted at Shayler Run in Ohio. The dominant species of algae in both the winter and summer was severely impacted by copper additions to this stream. Even though this occurred, algal growth of other species compensated and richness increased. As I recall no impacts on survival, growth, reproduction or community responses in fishes or

invertebrates was detected. Were the uses of this stream impacted? This question may need to be answered if phytoplankters prove sensitive in south bay waters.

SEM/AVS Ratios: This endpoint, along with interstitial water metals concentrations, must be one of those accepted as an indicator. The report indicates that no data are available on SEM/AVS ratios in sediments from south San Francisco Bay. Only metals concentrations on a dry weight basis have been measured. Dry weight metals concentrations have no value in rejecting or accepting metals as a cause of sediment toxicity.

Sixty-three percent of the south bay sediments used in toxicity tests were lethal to exposed organisms. What is the cause of the observed toxicity? An SEM/AVS ratio of <1.0 and the sum of the interstitial water toxic unit for metals less than about 0.5 can be used to demonstrate that metals are not the cause of toxicity in toxicity tests and in field sediments. Guidance on how and when to sample sediments is available from EPA. In addition, whole sediment TIEs can be used to determine if sediment-associated metals are the cause of toxicity. Given the high incidence of toxic sediments it would be a mistake to ignore these new techniques for excluding, or causally linking, metals in sediments to toxicity. Excluding metals-related sediment toxicity is a must prior to a decision that there is no impairment, or that impairment is unlikely, due to metals in south San Francisco Bay.

Charismatic Macrofauna: I agree with the non-inclusion of charismatic macrofauna in the list of indicators. It seems likely that there might be more data on doses of concern that could have been linked to dietary uptake by these predators. Given what was presented in the report, it appears unlikely that metals are affecting seals or birds.

Individual Species Toxicity Tests: I agree with the reports conclusion that these clean water toxicity tests, and WQC that result from them, are appropriate for developing indicators of the health of the bay as it pertains to the possible effects of copper and nickel. Toxicity tests have a very long history of scientific and regulatory acceptance. At this time, the joint use of WQC and site-specific WERs are the best approach for establishing site-specific WQC that account for the site-specific biological availability of metals. The biotic ligand model, which is inherently site-specific, will soon become acceptable as a replacement for the standard WQC approach and needs to be discussed at some level of detail.

EPA accepts, and many others believe that, the deletion of non-resident aquatic species from the WQC database provides an improved indicator of the sensitivity of local organisms over that of the entire WQC database. I believe that this deletion most often merely removes data from the national data set that likely represents the sensitivity of untested resident species. Further, the deletion artificially lowers the WQC by the reducing in the number of GMAVs used to calculate the FAV. If the report continues to use this deletion process, the rules for deletion in the "WER Cookbook" must be followed. These rules are specifically designed to limit the probability of deleting data on non-resident that are surrogates for untested resident species. These rules were not followed in this Tetra Tech document.

AERAP: This approach is intriguing because it combines databases from all aquatic toxicity tests with phytoplankton, macroalgae, macroinvertebrates and fish. This creates the impression that the AERAP approach is more ecological than the WQC approach. The Review Committee was not previously aware of this methodology published by WEF. Reports detailing the approach were not readily available to the committee. While the approach may have merit, there are concerns that need to be addressed before the method is adopted as a site-specific objective (SSO). These include:

(1) SSOs derived using this method seemed to result in unnecessarily low SSOs concentrations given that there was a sufficient number of taxa tested so that the 95% level of protection should have been within the toxicity data for the 34 species rather than at a concentration well below that of the most sensitive species. It is for this very reason that the WQC FAV calculation procedure was developed.

(2) The confidence limits on the species sensitivity distributions were narrow at the tails of the distribution where the data were limited and widest in the middle of the distributions where the data were most robust. This result is different from confidence limits of typical statistical analysis and needs to be critically examined. What are the assumptions of the statistical model? Must the data be from a randomly selected group of species, or can these biases of these data sets be used?

(3) The report must be clear that the distributions of species sensitivity and calculations of protection levels apply only to the data set of tested species. The report is wrong in stating that they can be directly applied as levels of protection for species in south San Francisco Bay.

(4) As the exposure concentration increases affecting more and more species, the species to species interactive effects would be expected to begin. Once this occurs, guessing the probable impacts based on the simple distributions of individual species sensitivity becomes problematic. This is one of the reasons why the level of protection must be high.

 (5) Application of ACRs derived from tests on acutely sensitive species must not be applied to insensitive species. While there are insufficient ACRs for saltwater species to show a trend in ACRs with acute species sensitivity, ACRs for freshwater species increase with decrease in acute sensitivity. If true for saltwater species, the use of the ACR from acutely sensitive species with acute values for acutely insensitive species will result in predicted chronic safe concentrations that are too high and these species will be under protected. The use of only the acute toxicity data in the logistic approach, as suggested at the meeting, may have merit in overcoming this short-coming . However, inclusion of phytoplankton and *Champia* data in this acute lethality database is inappropriate as endpoints are for growth rate or reproduction and not lethality. Even if this approach is used, reservations 1 and 2 need resolution.

(6) An alternative to the AERAP approach that would not mix toxicological oranges and apples would be to plot the acute sensitivity of fishes and macroinvertebrates vs rank. Indicate probable

chronic toxic concentrations using the ACR for only the few most sensitive species. Co-plot as a separate distribution on the same graph using a different symbol the rank sensitivities of plants including phytoplankton and macroalgae. Also co-plot with another symbol the data the WQC document refers to as "other data". Include the site-specific dissolved metal concentration (SSO) that is protective. A final shaded area could be added to represent the range of observed dissolved concentrations. These dissolved concentrations might be "corrected" for available metal by dividing by the WER. This approach would give the impression of the AERAP that it is "ecological" without mixing tests whose endpoints are toxicological oranges and apples and implying that they are cherries.

(7) Dividing the results of all toxicity tests by the site-specific WER has similar problems to the universal application of ACRs. WERs are highest for the most sensitive species, and likely approach 1.0 for insensitive species because the proportion of free metal increases with total concentration increase. Applying ACRs and WERs derived from tests with acutely sensitive species to insensitive species will result in under protective concentrations.

(8) Deletion of data from tests with "non-resident" species, if done at all, should follow the WER Cookbook.

Site-Specific Studies: The site-specific studies conducted by the City of San Jose, along with those conducted in the New York Harbor study, are the best that I have ever had the chance to review. The report uses these studies in an acceptable fashion. As mentioned earlier, I am concerned about the non-resident species deletion process used in the report. Certain reservations concerning the nickel site-specific study are identified in the "Specific Comments" section of this review.

Phytoplankton: This was an extremely clear and well written section. I endorse the need for additional studies on possibly cyanobacteria, cocolithophores, dinoflagellates, and diatoms to confirm their apparent unique sensitivities in certain laboratory waters. There is a need to determine if these algae are more sensitive than the species used to derive the various SSOs. I believe that others on the review committee will discuss the appropriate tests in more detail. Also, before tests begin it might be useful to have the study design reviewed. The idea here is to do the tests once to answer the question of concern.

Question 4: Do the findings and recommendations in the report follow logically from the data and scientific information presented?

Answer: The review Committee was unsure as to which of the many findings and recommendations in the report this question pertained. Without further guidance an answer is not possible. Never-the-less, the comments contained in this review should provide some insights into the answer to this question.

Question 5: Based on available evidence, can the null hypothesis that Cu and Ni impairment in the lower south San Francisco Bay exists be rejected?

Answer: For fish and macroinvertebrates the effect concentrations in water, and the SSOs, are greater than the site-specific measured dissolved concentrations. This indicates that effects of copper or nickel on these organisms appears unlikely. Concern remains because water quality models have not been run to confirm that when ambient concentrations are extrapolated to the WQC averaging periods and return frequencies no exceedances exist. Further, the fact that 63% of the sediments tested against benthic organisms have been lethal is disturbing. The good news is that there are definitive tests that will eliminate metals as a source of this toxicity. Finally, the sensitivities of certain phytoplankters to copper requires definitive tests using site water to determine if they are as sensitive in the waters of south San Francisco Bay. If the algae prove extra sensitive, an analysis of present ecosystem health would be invaluable.

Scientists always seem to need one more series of experiments to reject a hypothesis. Scientific review committees are even more cautious. It is not the role of individual scientists or scientific review committees to answer this kind of question. This is why there risk managers. If I was one of the managers I would want the small amount of effort suggested above completed, and then I would decide.

Question 6: The report describes a technical basis for establishing site-specific water quality objectives for copper and nickel at several different levels within an overall range. In your opinion, which specific concentration value for copper is best supported by the technical evidence and why? Please answer the same question for nickel.

Answer: I am biased, and my answer is based on that bias. As a former employee of the U.S. EPA I served on the WQC Guidelines Committee and as technical coordinator for saltwater WQC derivation. The WQC Guidelines Committee was charged with developing national and site-specific WQC derivation methodologies. These methodologies result in one number being derived. (This approach actually develops acute and chronic concentrations, an averaging period and a return frequency rather than a single number. There are reasons why all must be derived.) Therefore, I prefer an approach that uses all the available data to recommend just "one number". Multiple numbers give the impression that they somehow describe the uncertainty of the SSO derivation, but this is unlikely. Further, I do not know what to do with multiple numbers, and I'm quite sure that managers have the same problem.

Rather than directly answering the question with my best number, I will describe the approach I prefer. This is required because the devil is in the details. If I select from the various numerical SSOs in the report, it will mean that I have reviewed all of the data and data manipulations used in their derivation and in total agree with them. I did not do this. Therefore, I will specify the approach I prefer for derivation of the number: (1) The national WQC guidelines must be followed. (2) All available data that is acceptable according to these guidelines must be included. (3) Deletion of nonresident species is not recommended. (However, if the TMDL Work Group feels they must do this, the rules in the WER Cookbook should be followed.) (4) The value that results from the first three steps should be adjusted for site-specific water quality conditions using the WER(s) that apply. For copper, the WER(s) most applicable are from the City of San Jose studies. I prefer the use of all four WERs derived in the San Jose study. For nickel, the

WER data do not follow what should occur given expected metal speciation and its toxicological implications. I would have to conduct a greater review of these data before I could endorse their use.

Additional Questions:

Question 1: In the report, four Environmental Risk Concentration Values (ERCs) are presented, which are calculated using the AERAP model from four different toxicity databases. The ERC based on the National database is very similar to (but slightly higher than) the ERC based on the National/No plants, even though laboratory toxicity tests show that some species of algae, including *T. pseudonana*, are among the most sensitive organisms to copper. In developing criteria, EPA calculates a final acute value (FAV) from the four most sensitive species, divides by an acute-to-chronic ratio to arrive at a CCC. In the Report, the ERCs are treated as equivalent to a CCC in calculating possible site-specific criteria (i.e. they are multiplied by the WER). In developing a criterion that is protective of plants, is it more appropriate to use the AERAP or FAV method?

Answer: The key issue here is which method protects plants. The answer is that both protect plants because both directly consider the sensitivity of plants as summarized in the WQC databases. The AERAP includes the data on plants in the database used to calculate the ERCs and the WQC guidelines approach calculates an FAV and FCV and then compares these values to those for plants to assess the level of their protection. The concern raised in this question revolves around data that neither approach considered. These data suggest that the sensitivities of certain cyanobacteria, cocolithophores, dinoflagellates, and diatoms are unique. The degree to which these data apply to these phytoplankton in south bay waters can not be resolved until the special studies are conducted.

Question 2: Please evaluate (1) the level of conservatism of each of the key technical assumptions leading to the SSO (e.g., resident/surrogate species selection, AERAP % species protection, ACR value, 2 vs 3 station WER (i.e. geographic extent) and (2) the cumulative impact of these individual assumptions on the conservatism of the resultant SSO.

Answer: I do not know how to answer this question. There is no doubt that depending on what options are selected for the choices listed the SSO concentration is increased or decreased. If the choice is to always decrease the concentration the SSO will become lower/more conservative. To me the more relevant question is what choice is the most appropriate given the data and the scientific decisions required. I prefer using all of the available toxicological data so do not like deletion of any data. Even species not from the bay have relevance to untested bay species. Selecting the appropriate level of protection is important. AERAP selects multiple levels, but all are outside the database or fail to fit within the database at the appropriate percentage. Again I prefer using all available data, therefore, the FACR should be the geometric mean of the species, or genus, acceptable ACR values for acutely sensitive species. I actually prefer using all of the four WERs derived by the City of San Jose as applicable to the sites where they were derived and using the four site-specific WQC as targets for the waste load allocations.

Questions Related to Uncertainties and Special Studies:

Question: Have the most important uncertainties been identified?

Answer: Yes! The report and my comments contain the key uncertainties that pertain to the assessment of risks of copper and nickel to the south San Francisco Bay ecosystem.

Question: To what extent will the proposed special study reduce or eliminate the uncertainties.

Answer: There will always be uncertainties related to the presence of metals in the bay. At issue is will special studies on algae, metals bioavailability from sediments and water quality modeling to determine if SSOs (WQC concentrations) are exceeded given their respective averaging periods and return frequencies. I believe that if these studies continue to indicate that copper and nickel do not pose risks, managers with minimum risk will be able to reject the null hypothesis that Cu and Ni impairment in lower south San Francisco Bay exists.

Specific Comments:

- Bioaccumulation is exposure assessment not effects assessment. It is not necessarily an indicator of bioavailability that is relatable to effects. For example, the effects of metals in sediments is not correlated with dry weight metals concentrations, but many studies have shown that tissue concentrations increase with increase in dry weight metals.

-WQC consist of an acute and a chronic concentration, an averaging period and a return frequency. These are absolute requirements that permit calculation of TMDLs. The Report is dedicated to the derivation of the chronic concentration, but does not mention the other components. When I asked about this the response was that consideration of the averaging period and return frequencies was not needed because effect (SSOs) and exposure concentrations never overlapped. Only proper water quality models can demonstrate that the distribution of the water samples is such that if modeled over many years an exceedence would not occur. This should be done.

-The Glossary of terms does not follow the definitions in the context of WQC derivation. The FAV definition is not correct and there is no discussion of how the CCC is derived.

-Watson et al. (1996; 1999) reports on the recalculation of the nickel WQC were not provided so the "questionable data" that were deleted can not be reviewed. The idea that a range of a factor of three in ACRs suggests that the CCC for marine species is overprotective is wrong. ACRs can vary by over a factor of three even in replicate tests in the same laboratory. This is because they include the variances of both acute and chronic tests.

-Much of the discussion of uncertainties in 4.1.5 misses the point. The issue is not the sources of uncertainty inherent in toxicity tests. Instead the critical uncertainties are in their use in extrapolation to predictions of site-specific effects. While there has been a significant effort

directed at this, the magnitude of this uncertainty is highly debated. I believe that most would generally agree that given the same exposure conditions, responses of individual organisms would likely be similar in the laboratory and the field. Next the uncertainty that needs estimation is that associated with the use of toxicity data to derive WQC or AERAPs and the uncertainties associated with their derivation and site-specific applicability. Some of the sources of uncertainty are inherent in the required toxicity databases and their extrapolations; i.e., test to test, species to species, life-stage to life stage, acute to chronic, etc. Finally, the uncertainties related to the application of laboratory derived WQC to the field when carefully studied in studies like those at the Monticello, MN channels indicates that WQC concentrations are protective.

-There needs to be at least a limited discussion of the biotic ligand model (BLM) that links water quality models for metals to the gills, or other critical tissues, as another ligand to be modeled. Once the critical concentrations at the site of action is known, the model can utilize site-specific speciation of the metal to determine if effects might occur.

-The freshwater toxicity data on salmonids are useful. The toxicity of metals to salmon in saltwater should be less than the toxicity in freshwater, particularly fresh waters with low hardness. Therefore, if salmon tested in freshwater are not sensitive at SSOs for saltwater, then they will likely be protected.

-Stephan et al., 1985 had nothing to do with site-specific WQC.

-The description of the Indicator Species and the Resident Species Procedures does not capture the complexity of this methodology and in parts is wrong.

-Table 4-7. How could the acute values be exactly the same in site and laboratory water? ACRs are not calculated based on EC50 values. The EC50 values for the two of the four chronic tests are surprisingly similar. Was this just good replication or an error?

-Table 4-8. Why were EC50 values not used to calculate the WER? WERs should decrease with less sensitive species. Dividing the EC50 for bivalves by two to estimate the chronic value is no longer acceptable.

-Table 4-9. The text says the two species were selected because they were most sensitive yet the WERs differed because sensitivities differed. Explain! Something is wrong as the most sensitive species had a WER less than 1.0 and the less sensitive species the WER of about 10.

-p.4-52. Tests with early life stages are not equivalent to early life-stage tests. Early life-stage tests are equivalent to chronic life-cycle tests.

-Did Tetra Tech consider adopting four different WERs to represent the four stations for which WERS are available.

-Explain in the report why the AERAP procedure was not used with nickel.

-Were the FACRs calculated using GMACRs or test by test ACRs?

Comments from Jonathan Phinney

Jonathan T. Phinney Ph.D.
8503 Doter Drive
Alexandria, VA 22308

September 24, 1999

To: Tom Grieb, Tetra Tech
From: Jonathan Phinney
Re: Impairment Assessment Report Technical Review

Tom,

Here are my answers to the specific questions posed by the TMDL Work Group Subcommittee as well as general comments and suggestions for the report.

General Observation

The report is very through both from a regulatory and scientific standpoint. Having to straddle those two realms in my present position, I appreciate the magnitude of the task and give the technical consultants high marks for completeness. In my opinion, the Cu and Ni values calculated for the South Bay are conservative and protective of multicellular organisms. For single cell phytoplankton, cyanobacteria and larvae (shellfish and finfish), the consensus of the technical review committee was that more information is needed including scientific studies that is explained in more detail below.

Answers to Specific Questions from Technical Review Committee's Review of the Draft Final Impairment Assessment Report.

(Reference: e-mail from Jerry Boese to tmdl@egroups.com, sent Thursday 9-09-99 11:03 AM)

1) Is the method or approach for evaluating impairment in the Report reasonable?

From a scientific standpoint, the assessment of impairment seems complete and reasonable. Focusing on dissolved Cu and Ni concentrations and not the labile metal (sum of free and inorganically complexed metal) is conservative and precautionary as it should be. The strategy developed (identification and evaluation of indicators, compilation and evaluation of ambient conditions, quantification of uncertainty and development of a range of metal concentrations) is valid. While there is little information on the bioavailability of particulate metal on macroorganisms, I would caution not to focus only on the dissolved fraction. At the very least, bioavailability of particulate metals to fish and macroinvertebrates is not well understood and should be acknowledge in the report. The book, Trace Metal Speciation and Bioavailablity edited by P.G. Campbell and A. Tessier could be consulted to review this topic.
Are all the relevant data included or summarized adequately in the Report?

Yes. There is a plethora of peer reviewed scientific articles that overwhelmingly demonstrate the applicability of the "free ion activity model" for trace metal toxicity (reviewed by P.G. Campbell 1996 in <u>Trace Metal Speciation and Bioavailability</u> -see #1). There are a few exceptions to the free ion model that should be mentioned in the report. In particular, lipophilic organic metal complexes that diffuse across membranes should be better developed in the report (reviewed in Campbell 1996). To date, one field study has suggested that lipophilic organic Cd complexes may exist in fresh water systems. There are no similar marine studies. However, IBM and the airplane repainting hanger at the SF Airport were experimenting with Betz[tm] ligands in 1996 to complex metals in their wastestreams. (I don't know if they are still using the ligands, and Wayne Young at IBM could give the present status.). These ligands are very similar to dithiocarbamates ligands that can form lipophilic Cu and Ni complexes that diffuse across phytoplankton cell membrane (Phinney and Bruland 1997 in report). There is no evidence that these ligands make it through the wastewater treatment process at the facilities and into SF Bay. Nonetheless, I would suggest consulting the two facilities to see if they still use the ligand.

3) Does the discussion of indicators reflect the current state of scientific knowledge on the relevant topics?

The indicators used (Individual Species Toxicity Tests; Aquatic Ecological Risk Assessment, Protocol (AERAP), Site Specific Studies Indicator, and Phytoplankton) are current. In consultation with the Technical Review Scientists, I would add that benthic macroinvertebrates and sediment tests not be discounted completely. Dr Kuwabara mentioned that the USGS may have sediment data and benthic invertebrate data in the South Bay. If there is benthic macroinvertebrate data available, there is a benthic index that was developed for the Chesapeake bay that could be utilized. (It was developed by Vesar Consultants in Columbia, MD and is a part of the Chesapeake Bay Program protocol. Contact Kelli Eisenman (410 267-5700) at the EPA's Chesapeake Bay Office in Annapolis, MD for more information).

On the scientific side, the discussion of labile metals (free and inorganically complexed fraction) and competition between metals (and the lessening of toxicity) are also current. Lipophilic organic metal complexes should not be discounted in light of potential increased uses mentioned in the last question. Kinetics especially of organic Ni complexes is covered in Bedsworth and Sedlak 1999 and should also be included in the report as additional evidence for a low concentration of labile Ni. Use of Metal' (the sum of the free and inorganically complexed metal, e.g. Cu' and Ni') is more appropriate than free metal as the bioavailable fraction. One can't distinguish between the free and inorganic metals since the reaction kinetics are very rapid. The focus on dissolved fraction is conservative and a good first approximation of whether toxicity could be present.

Do the findings and recommendations in the Report follow logically from the data and scientific information presented?

The technical review committee needed more specifics on what findings and recommendations needed to be reviewed.

(After the technical review committee meeting, the author of this question said to me that the this question was no longer relevant and that question 5 would suffice. I asked her to relay that message to Tom Grieb).

5) Based on available evidence, can the null hypothesis that Cu and Ni impairment in the lower South San Francisco Bay exist be rejected?

For macro and mega sized organisms I feel that the null hypothesis can be rejected. For single cell organisms (phytoplankton, bacteria and larva) that comprise the basis of the foodchain, there is still scientific uncertainty about whether impairment exists. Because of their position in the foodchain, I feel that it is very important to lower the uncertainty for these organisms by conducting additional scientific studies. Figure 4-12 (Range of pM^+ values in oceanic and estuarine environments) is very effective at summarizing the debate of whether the microorganism are impaired in the South Bay. The pCu concentrations measured by Donat et al. 1994 are greater than the toxic concentrations measured by Brand et al 1986 (both cited in the report). The Donat et al. study had only two sampling trips which is a small sampling size. Also pMn concentration and pZn have not been measured in the South Bay.

Cyanobacteria (blue green algae) are bacteria and are the most sensitive species for Cu toxicity (Brand et al. 1986) and the first set of experiments should determine whether cyanobacteria growth are impaired in South Bay water. Cultures of cyanobacteria can be obtained from Bigelow Laboratory, Boothbay, ME (or other culture centers). Water from multiple sites (at least three) in the South Bay during the dry and wet periods should be built into the experimental design.

If there is no toxicity to cyanobacteria in these experiments (and there is proper statistical rigor in the design), then I would support rejecting the null hypothesis. If there is toxicity in these experiments, then I would conduct speciation studies on Cu, Zn, Mn, and possibly Fe speciation studies to measure the inorganic metal (which includes the free ion) to determine whether trace metals are responsible for the toxicity.

The weight of evidence does support the null hypothesis. However, there are experiments that need to be completed before I am comfortable rejecting the null hypothesis.

6) The report describes a technical basis for establishing a technical basis for establishing site-specific water quality objectives for Cu and Ni at several different levels within an overall range. In your opinion, which specific concentration value for Cu is best supported by the technical evidence and why? Please answer the same question for Ni.

I don't feel that I can provide a better site specific Cu and Ni value than what is proposed in the Impaired Assessment Report. I am comfortable that the process developed by Tetra Tech for establishing site-specific water quality objectives for Cu and Ni are adequate and protective of South Bay water. The technical advisory group and in particular Dave Hansen had questions about the use of the AERAP protocol. I will defer to his opinion about its efficacy.

IV Questions related to the Uncertainties and Special Studies

1) Have the most important uncertainties been identified?

Yes. I would add the uncertainty of bioavailability of particulate metals to megafauna that has not been adequately addressed and a benthic macroinvertebrate index should be addressed.

2) To what extent will the proposed special study reduce or eliminate the uncertainties?

Uncertainties regarding Cu and Ni toxicity will never be eliminated. The chemical cycling and biological effects are very complicated to discern. In my opinion, the uncertainties including the effects of competition from other metals especially Mn and Zn and the extent of organic complexation can be reduced substantially with the proposed speciation and toxicity study using phytoplankton (I presume although it is not explicitly stated). To reiterate an earlier point, the study should include toxicity tests in South Bay water using the most sensitive species known, cyanobacteria.

Characterization of the organic ligands in the South Bay is another study with merit, although the analytical methods are still being developed. While measurements of synthetic ligands such as EDTA have been done in the South Bay (Bedsworth and Sedlak 1999 in report), it is not certain that organic metal complexes (except $NiEDTA^{2-}$) can be measured and therefore modeling will play a potentially large role.

Determination of Cu and Ni in phytoplankton cells as an estimate of biological effects will be complicated by the fact that phytoplankton can adapt to high metal concentrations by producing phytochelatins that sequester the metal from the cell. So having high intercellular Cu and Ni concentrations will not be a good surrogate for toxicity.   A better approach would focus on measuring stress proteins (e.g. P_{450} and/or phytochelatins) as a measure of phytoplankton health rather than only total cellular concentrations.

 ADDITIONAL QUESTIONS SENT THR 9-09-99 (relevant pages 5-13 to 5-16, Tables 5-3, 5-14, Fig 5-1)

For Cu criterion development, is the AERAP(Aquatic Ecological Risk Assessment Protocol) or FAV (Final Acute Value) method more appropriate to use?

Dave Hanson expressed reservations about the AERAP protocol, and I defer to his comments about the efficacy of using AERAP or FAV.

Both AERAP and FAV provide conservative values for Cu criterion and are a first approximation of a potentially toxic Cu concentration. It is very likely that the laboratory toxicity tests that both criterion use are limited in applicability because they focus on toxicity of a single metal and do not know the extent of competition between metals or complexation of metals.

Evaluate the level of conservatism of key technical assumptions leading to the

SSO (Site Specific Objective using resident/surrogate species selection).
AERAP % species protection.
ACR value.
2 vs. 3 station Water Effects Ratio (WER).
Cumulative impact of these individual assumptions on the recommended SSO.

My opinion is that the technical assumptions in the report are highly conservative and the cumulative impact of these assumptions on the recommended SSO is low.

SPECIFIC COMMENTS ON THE REPORT

Section 2-2. The use of the term "indicators" needs to be better defined. In some cases, indicator species are being examined and at other points indicator tests or criteria are used. Table 2-1mixes these two categories up: #'s 1-4 (in Table 2-1) refer to indicator species for evaluation; #'s 5-11 are indicator criteria.

Glossary of terms in one place rather than Table 4-4, 4-28, etc.

Page 2-6 Benthic macroinvertebrates should be reassess to determine applicability for impairment and not discounted. They are used extensively in the Chesapeake Bay Program's assessment of toxicity in the Bay and there is a benthic index that was developed.

Page 2-6. SEM/AVS studies in the South Bay would be beneficial. I don't know of any data on the depth of the anoxic sediment in the mudflats areas where winds and currents can cause massive resuspension of sediment. I don't feel that a West Coast validation of the procedure is necessary is the processes involved (binding of sulfides with metals and lowering toxicity) are common in all sediment.

Page 2-6; paragraph 1 lines 6-7 states that "plankton (are) not recommended as a primary indicator in the assessment". However Section 4-4 develops a good rational for assessing plankton. Population dynamics of phytoplankton are very difficult to conduct and assess as stated in the report. But I would propose that if there are no adverse effects on the single cell phytoplankton with the greatest surface area to volume ratio (and thus the most susceptible to waterborne toxins), then the case can be made that Cu and Ni are not affecting the South Bay system.

Page 2-10, paragraph 4, bullet 3 needs better explanation about why a direct linkage between the concentrations of Cu and Ni and bird populations. Is it because of migration patterns for birds? Are there resident bird populations that could be used?

Table 3-1 could use a map to demonstrate where the stations are located.

Page 3-7, line 7, "relatively small" coefficients of variation needs to be quantified.

ibid line 7, "relatively low variability' of the Total and Dissolved Cu and Ni coefficients of variation is contrary to the data given. If I correctly understand the table, Total Cu concentrations ranged from 22-126 %. That is high.

Page 3-5. Paragraph 5, lines 8-10. The Ni evidence here is very important and demonstrates the relative inputs from nonpoint sources. I would highlight this information more in the site specific criterion section.

Section 3.3.1 page 3-11 bullet 1 explains that Cu and Ni are excluded as potential toxicants because the dissolved concentration were lower than toxic thresholds reported (in the literature). These values are dissolved concentrations and not pCu and pNi. Without these measurements, you can't rule out Cu and Ni completely.

Page 3-14 last paragraph. I would like someone to reaffirm the QA/QC methods used in the Larry Walker 1991a and b studies. The RMP measured toxicity in 60 % of the sediment samples whereas Walker's tests indicate the sediments are no more toxic (needs quantification as to what "no more" means) than other sediments in the Bay.

Section 4.0 and the indicator development is very confusing because there is no distinction between indicator species and tests as mentioned in #1 above.

Page 4-1. 2 paragraph, line 8. Add "in situ" phytoplankton studies, while…" rather than "The phytoplankton, while…". In situ studies have many uncertainties associated with them. Laboratory experiments with phytoplankton can be very definitive about toxicity.

Ibid., paragraph 5 "The following sections…" . Add "test" or "test for indicator species" at the end of sentence.

Page 4-2, paragraph 1, line 1, "'clean' laboratory water…". I question the "clean" procedures for the aquatic toxicity bioassays tests used and suggest a review of the procedures. Many of the bioassay tests mentioned in Appendix C were conducted in the early 1970's and early 1980's. "Clean techniques" for measuring environmental samples were first done in the late 1970's (e.g. Franks and Bruland 1978). Laboratory experiments such as Sunda and Guillard 1976 (in report) used high concentrations of EDTA and other chelators to bind trace metals and not "clean" techniques.

Page 4-4, bullet 1 (NOEC…), line 2. I would add that the organisms referred to here are macro and not microorganisms.

Page 4-10, paragraph 1, line 3 "ions". Add "inorganic ions" rather than ions.

Page 4-11, paragraph 2, line 1-2 "Resolving This Uncertainty-….". I would argue that algae are not used to set water quality criteria because of lack of perceived economic value NOT because of "difficulty in interpreting the results". As mentioned, the high surface to volume ratio of algae make them better indicators of water-borne toxins than multicellular organisms.

Ibid. Lines 2-4. Filtering water and adding nutrients don't have to alter the water conditions. If the metals are strongly complexed to EDTA, then filtering the water would do nothing to the organic metal complex. If the metal is bound to humic substances that can be altered by filtration, then I would suggest that this organic metal species does not adequately protect the organism and should be considered a potentially toxic species. The effects of nutrient additions are presumably due to the addition of trace metal contamination to the solution. This can be minimized by the quality of the reagent and the dilution factor built into the experiment- i.e. add a small quantity of nutrient stock solution to a large volume of culture.

Page 4-14, Section 4.2.2, lines 6 and 8. The terms "adequate" and "sufficient" need to be better quantified.

Page 4-24, Section 4.2.5, last bullet. I would argue that in situ toxicity tests with cyanobacteria would be the most definitive determination of whether a problem exists in the South Bay.

Page 4-27, Section 4.3.2, line 4. Inorganic ligands (e.g. CO_3^{2-}, CL^-, can not be distinguish from the free ion concentrations and should be considered a portion of the bioavailable fraction.

Page 4-47, paragraph 3, line 11, "reduced-toxicity complexes" should be changed to organic Cu complexes that are less bioavailable and therefore less toxic".

Page 4-52, paragraph 3 and bullets. The uncertainty and resolving the uncertainty bullets listed are identical to those listed on page 4-9. I would not repeat these verbatim, but make a table listing them and referring to the table in the text.

Page 4-57, last paragraph, line 4-5 ("Depending on circumstances…"). What circumstances are referred to here?

Page 4-59, last paragraph, line 1. Change "functions" to "proportional" to the free ion concentration.

Page 4-61, last paragraph, line 1. As mentioned above, "'clean' water" should not be assumed for studies conducted in the 1970's and early 1980's as listed in Appendix C.

Page 4-73, Section 4.4.6, paragraph 1, line 1, "more data gaps". The analysis for using phytoplankton as indicators of toxicity is well developed in the section. I would argue that this indicator has better data to determine potential toxicity than the others listed. The free ion activity model was developed using phytoplankton and there are established analytical techniques to measure the free ion species.

Page 5-8, paragraph 1, line 16. "slow kinetics" refers to Ni only and not Cu.

Page 5-14, paragraph 3, line 6. There are too many significant figures in the 3.127. I realize that this is the number from EPA guidelines, but it denotes a certain accuracy that is not inherent from the data. I would round up to 3.13 to be conservative.

Comments from Jim Kuwabara

Technical Review of Impairment Assessment Report

J.S. Kuwabara

Page 1-1, paragraph 2, line 15.

How does one link the toxicological and geochemical focus of this report to the forcing functions that physically affect the distribution of Cu and Ni in the South Bay (i.e., How do we link this work with results of the Conceptual Model, Sources, and Hydrodynamic Modeling Reports?) This clarification may be necessary to go from impairment assessment to SSO recommendations. There is a conceptual leap between determining whether there is evidence of resource impairment, and determining recommended values for site-specific objectives. The latter integrates information about processes discussed in other reports.

Page 1-3, paragraph 3, line 1.

For whatever reason, the Basin Plan does not consider that the South Bay may generate beneficial uses as a solute-transport and reaction conduit (relative to other waste discharge strategies). It would seem that this omission makes it more difficult to view the establishment of water quality objectives as a prioritization of the beneficial uses.

Page 1-4, paragraph 1, line 1.

In terms of the Basin Plans reference to the Lower South Bay, it would seem that all aquatic environments are "water-quality limited". However, as your synthesis of information has indicated, the relative importance of certain physical, geochemical and biological processes make the system to unique. For example, the first paragraph indicates the importance of South Bay hydrodynamics. It would follow that a appropriate development of site-specific objectives, TMDL's and waste allocations should consider the broad scope of those processes as can be integrated from the series of interdependent technical reports. It may be useful to clarify in this or subsequent sections how this integration of process information is made to establish an "integrated assessment" as mentioned in section 1.3 (page 1-5).

4.   Page 1-6, paragraph 2, line 5.

To be consistent with the bullets on page 1-1, should the "Potential Outcomes" of the assessment include recommendation of numeric values for SSOs? It appears to be the ultimate outcome of the assessment.

5.   Page 2-1, paragraph 3, line 8.

Probably want to include solution-phase concentrations if you are going to include sediment concentrations.

Page 2-2, paragraph 1, line 2.

Suggest inserting the word "impairment" before "assessment feasible" for clarification.

Page 2-2, paragraph 3, lines 5-7.

Does this strategy need to include an attempt to understand what generates those ranges (uncertainties) in the SSOs? If not, how does one select appropriate SSOs from within those ranges?

Page 2-6, paragraph 1, line 15.

As mentioned at the Review Committee meeting, you might consider the implications of the Cu/Si interaction as reported by Rueter et al (1981; reference provided at the meeting) to the diatom-dominated South Bay phytoplankton community. One might ask, "Does elevated dissolved silica concentrations (70-120 uM) have an antagonistic effect on copper toxicity to diatoms in the South Bay?"

Page 2-6, paragraph 3, line 12.

I would speak to scientists who have done this type of macro-invert work (e.g., Bruce Thompson, Sam Luoma) to get references that would support the assertions made in this and the next paragraph, particularly about the difficulty in identifying and parameterizing causal effects.

Page 2-7, paragraph 4, line 1.

As mentioned at the meeting, you might check with Byeong Lee (650-329-4466) about his AVS-SEM studies in the South Bay. It may provide a better basis for the statements made in this section.

Page 3-7, paragraph 3, line 2.

In this analysis, how does one discriminate between solute exchange due to sediment resuspension, and exchange due to biologically enhanced advection? I think we all agreed that the kinetics of trace-metal adsorption/desorption reactions are poorly understood. There is also evidence (e.g., Jan Thompson's work, the reference I gave Tom, and other work by Kenneth Coale and Will Berelson) that diffusion control of solute benthic flux may not be a reasonable assumption.

Page 3-8, paragraph 1, line 3.

Sediment concentrations for Cu and Ni seem from the table to be relatively constant, but you correctly pointed out at the meeting that there is a paucity of any of pore-water data or direct solute flux measurements (You might check with Kenneth Coale (Moss Landing Marine Lab, 831-755-8650) about trace-metal results from his ONR studies?

Page 4-2, paragraph 2, line 3.

Are you confident that these tests provide a "worst case"? Without the complexing capacity designed into the media, how is the bioavailable Cu concentration maintained during the bioassay. As mentioned in the meeting, chemically-defined media for such studies usually take the opposite approach so that transcient effects during the incubation are minimized. One needs to consider the possibility of decreases in total Cu and increases in complexing capacity (ligand exudation) in media where low complexing capacity is imposed.

Page 4-2, paragraph 3, line 3-4.

Does "total copper" here mean total dissolved copper? The modifiers "dissolved" and "total" are both used in this paragraph. Some brief clarification on their distinction would be helpful (maybe reference in the Glossary of Terms; p. 4-28).

Page 4-5, paragraph 3, line 2.

What accounts for the difference between the results of the San Jose WER Study with low WER variability, and the 1991/92 consultant studies with high WER variability. If it has to do with "totals" vs. "dissolved", that might be clarified in paragraph 4 as a link to the description of the saltwater criteria.

Page 4-6, paragraph 2, line 12-16.

Please reconsider the level of precision represented in these CCC estimates.

Page 4-9, paragraph 9, line 1.

As mentioned in comment 14, please consider that in these bioassays if pCu is buffered during the incubation. Maintaining a constant pCu in media with low complexing capacity is typically difficult in a batch experiment. This is apparent when one looks at the formulations for algal culturing media where metal speciation is critical to the experimental design (e.g., AQUIL and SANME media).

Page 4-10, paragraph 4, line 1.

The issue of resident versus surrogate species brings an associated consideration of species tolerance. Ken Bruland mentioned at the meeting that species from culture collections can be much more metal tolerant. The same could be true of strains that are collected from areas of the

bay were metal availability is elevated. Metal tolerance can be developed within a few generations for certain phytoplankton species.

Page 4-13, paragraph 3, line 2.

As mentioned at the meeting, it may not be reasonable to have 1 point represent the acute or chronic toxicity for a given species or genus. The cumulative frequency curve has other sources of error besides the regression. I understand that it is enticing to apply a protocol that looks at a predicted community response, but it would appear that the interpretation of the cumulative freqency has some major limitations. The general comment is a good one, that others who use the AERAP protocol should be consulted, particularly to the curve construction at the most sensitive end.

Page 4-16, paragraph 2, line 3.

The "Gold Book" reference does not appear on p. 7-7.

Page 4-22, paragraph 1, line 4.

Tom, the slide you showed about error propagation in generating SSOs was useful to see. Sources of uncertainties can be quickly seen.

Page 4-54, paragraph 3, line 4.

Full characterization of the ambient water would include studies on the kinetics of metal repartitioning (a major information gap recognized in the conceptual model).

Page 4-67, paragraph 2, line 7.

There may be more tolerant communities of some species. For example, there may be protectively high dissolved silica concentrations in the South Bay (Rueter et al., 1981).

Fig. 4-5

The deterministic representation of toxicity data for San Francisco Bay species is addressed by invoking a WER correction. It is not clear to this reviewer, but it maybe to others who apply AERAP techniques, how appropriate that statistical algorithm is (suggest adding a few supporting references about successful AERAP applications).

Page 5-8, paragraph 3, line 5.

Metal repartitioning rates as determined in adsorption/desorption experiments represent an important information gap in the interpretation of this sediment toxicity data.

Page 5-9, paragraph 1, line 2.

Insert "provide" between the words "may" and "procedures".

TRC Comments on Summary Documents

1.   Review Comments from Dr. Ken Bruland

From: Ken Bruland [bruland@cats.ucsc.edu]
Sent: Monday, December 06, 1999 4:26 PM
To: Grieb, Tom -- Tt, Inc.
Subject: Re: South San Francisco Bay TMDL

Tom,
I'm OK with the summary report of the reviewers comments.

Ken
I know we talked about your review of the Tetra Tech follow-up report that summarizes the comments of the Technical Review Committee on the Impairment Assessment Report, but I am unable to find a copy of any comments you may have sent to us. This is just the summary of the reviewers comments, and we want to make sure that we have captured the information correctly.

If you have provided any comments could you re-send them to me? Otherwise could you send a note indicating your judgment on the summary report?

Thank you

Tom Grieb

Professor Ken Bruland
Ocean Sciences Department
University of California at Santa Cruz
Santa Cruz, CA, 95064
Office: 831-459-4587

2.   Review Comments from Dave Hansen

From: Dhansen334@aol.com
Sent: Friday, November 05, 1999 5:42 AM
To: Tom.Grieb@tetratech.com
Subject: Re: File 1 as WordPerfect

I have reviewed the file entitled "report" received from you yesterday. The report by Tetra Tech summarizes the comments of the Review Committee and responds to the comments. The report accurately captures comments attributed to me. I did not review the file entitled "attachme" because it contains my comments concerning the meeting that I sent you previously. Thank you for allowing me to examine these files.

3.   Review Comments from Dr. Jonathan Phinney

<div align="center">

Jonathan T. Phinney Ph.D.
8503 Doter Drive
Alexandria, VA 22308

</div>

November 5, 1999
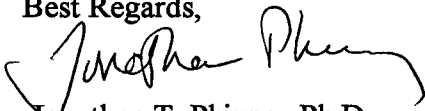
Tom Grieb
Tetra Tech
3746 Mt Diablo Blvd
Lafayette, CA 94549

Dear Tom,

I have read the report summarizing the Technical Review Committee's comment of the Impairment Assessment Report and feel that your group did a fair and accurate job. While I have a few quibbles on wording, there are none major enough to warrant additional clarification on your part.

I thought that the cyanobacteria data in the South Bay listed in the summary was very timely and important.   Because of their importance to determining whether Cu is toxic to phytoplankton, I would suggest that a small study be conducted this spring that would measure the number of cyanobacteria and Cu speciation. I can elaborate on study design and suggest researchers if you are interested.

Best Regards,

Jonathan T. Phinney Ph.D.

4.   Review Comments from Dr. Jim Kuwabara

From: kuwabara@usgs.gov
Sent: Wednesday, November 03, 1999 9:42 AM
To: Grieb, Tom -- Tt, Inc.
Subject: Re: South San Francisco Bay TMDL

Tom - I've had a chance to look over your email attachments and think that Appendix E and Attachment 3 generally provide a good summary of the issues brought up in the review.   In terms of formatting Appendix E, you might consider incorporating section 2.3 (Additional comments by the TRC) into the responses to questions 7 and 8 (pp. 14 and 15). For example, defining the linkage between the Impairment Assessment Report and Other Reports that describe contaminant transport in the South Bay addresses uncertainties that limit the application of toxicological (bioassessment) data.

Best regards. - Jim

---------------------- Forwarded by James S Kuwabara/WRD/USGS/DOI on11/03/99 09:38 AM

(Embedded image moved to file: pic16438.pcx)
James S Kuwabara
(Embedded image moved to file: pic28782.pcx)
11/03/99 09:06 AM

To: "Grieb, Tom -- Tt, Inc." <Tom.Grieb@tetratech.com>
Subject: Re: South San Francisco Bay TMDL (Document link not converted)

Hi Tom - Returned late last night, so got your email and phonemail messages this morning and will try to quickly look at the attachments today. - Jim

Appendix H
TRC Review Comments
4/17/00

APPENDIX H

Report to the TMDL Work Group on the Technical Review Committee Comments Regarding Phytoplankton Studies

April 17, 2000

A Technical Review Committee (TRC) reviewed the draft Final Impairment Assessment Report. Members of the TRC included: Ken Bruland, University of California at Santa Cruz; David Hanson, HydroQual; Jim Kuwabara, USGS; and Jonathon Phinney, Center for Marine Conservation. The report on this review process is presented in Appendix G. As part of that review, TRC members were asked whether "Based on available evidence, can the null hypothesis that Cu and Ni impairment in the lower South San Francisco Bay exists be rejected?" In response, TRC members reported that the null hypothesis could not be rejected without additional information on the toxicity of copper to sensitive phytoplankton (cyanobacteria) in the South Bay.

Since the TRC's review of the draft Final Impairment Assessment Report, additional information became available regarding the occurrence of cyanobacteria in San Francisco Bay. This new information is presented in Appendix I, and it asserts evidence that show that cyanobacteria were a "persistent component of the San Francisco Bay phytoplankton in all the estuarine habitats" in 1998 and 1999. In light of the new information, the TMDL Work Group requested that TRC members revisit the question of impairment (Attachment 1). Specifically, TRC members were asked the following questions:

- The TMDL Workgroup would like to know if the results from the two studies would lead you to modify your original assessment regarding the toxicity of copper to sensitive phytoplankton as well as your general conclusions regarding impairment.

- In addition, if you were to modify your conclusions regarding impairment, how would you change your recommendations for follow-on studies?

The responses of the individual TRC members to these questions are presented in Attachment 2. Overall the TRC members responded to the new information with guarded optimism. The reviewers agreed that the studies were sound and showed that cyanobacteria are a consistent part of the phytoplankton population in South Bay, however several unanswered questions remain.

The following is a summary of the TRC responses:

Question One: Do the results from the two studies lead you to modify your original assessment regarding the toxicity of copper to sensitive phytoplankton as well as your general conclusions regarding impairment?

The responses of the TRC ranged from a rejection of the original null hypothesis that Cu and Ni impairment exists in the lower South San Francisco Bay to acknowledgement of the

existence of at least one sensitive phytoplankton species in the these waters. As a group, the reviewers noted that while these new studies on the occurrence of cyanobacteria in lower South San Francisco Bay provide valuable new information, they do not resolve all the technical questions regarding the effects of free ionic copper on sensitive phytoplankton species.

Question Two: If you were to modify your conclusions regarding impairment, how would you change your recommendations for follow-on studies?

The Technical Review Committee offered several suggestions for additional studies. These suggestions included:

- Additional monitoring of free copper concentrations and better characterization of phytoplankton populations

- Evaluation of the adaptive mechanisms and acquired tolerances of cyanobacteria

- Evaluation of the nutrient metal interactions that may be reducing toxicity to Synechococcus and improved understanding of Cu-Mn or Cu-Si interactions in relation to impairment

- Additional toxicity tests to fully characterize the unique sensitivities of the full range of cyanobacteria species.

In summary, the TRC responses to the questions regarding the significance of the new information on the occurrence of cyanobacteria in lower South San Francisco Bay lend support to the finding that impairment to the beneficial uses of lower South San Francisco Bay due to ambient copper concentrations is unlikely.

DRAFT TMDL Work Group Memorandum

FROM: **Tom Grieb, Tetra Tech**

TO: **Ken Bruland, Dave Hansen, Jim Kuwabara, Jonathan Phinney**

DATE: **March 17, 2000**

SUBJECT: **Review of Recent Information on the Occurrence of Cyanobacteria in Lower South San Francisco Bay**

One of the questions that you addressed in the review of the Draft Final Impairment Assessment Report was "Based on available evidence, can the null hypothesis that Cu and Ni impairment in the lower south San Francisco Bay exists be rejected? In the Technical Review Committee Summary, it was reported that you agreed that this null hypothesis cannot be rejected without additional information on the toxicity of copper to sensitive phytoplankton (cyanobacteria) in the South Bay. Dr. Phinney in his written comments said "Cyancobacteria (Blue green algae) are bacteria and are the most sensitive species for Cu toxicity (Brand et al. 1986) and the first set of experiments should determine whether cyanobacteria growth is impaired in South Bay water."

In the Draft Final Impairment Assessment Report this information on copper toxicity to phytoplankton was summarized in the following statement: " Several studies have reported on the sensitivity of several classes of phytoplankton (cyanobacteria, coccolithophores, dinoflagellates, and diatoms) to free ionic copper. These classes of phytoplabnkton were found to exhibit reduced growth at free ionic copper concentrations as low as approximately 10^{-11} M with cyanobacteria being the most sensitive to free ionic copper concentrations followed in order of decreasing sensitivity by coccolithophores, dinoflagellaters, and diatoms."

Since your review, additional information has become available regarding the occurrence of the picocyanobacteria Synechococcus sp. in San Francisco Bay. The two attached papers[1] show that cyanobacteria were a "persistent component of the San Francisco Bay phytoplankton in all the estuarine habitats" in 1998 and 1999. In light of this new information, the TMDL Workgroup has requested that you revisit this question. The TMDL Workgroup would like to know if the results from the two studies would lead you to modify your original assessment regarding the toxicity of copper to sensitive phytoplankton as well as your general conclusions regarding impairment.

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[1] Ning, X., J. E. Cloern and B. E. Cole. 2000. Spatial and temporal variability of picocyanobacteria *Synechococcus* sp. in San Francisco Bay. Limnol. Oceanogr. (in press); Palenik, B. and A. R. Flegal,1999. Cyanobacterial populations in San Francisco Bay. Regional Monitoring Program for Trace Substances, Technical Report. http://www.sfei.org/rmp/reports/cyanobacterial.html)

1.   Review Comments from Dr. Ken Bruland

Dr. Bruland was interviewed via telephone and his responses were included in bulleted format and faxed to him for review and comment. His responses:

Dr. Bruland: Question 1 (telephone interview)
- Limited monitoring suggests seasonal variability with presence
- Populations could have adapted to local conditions
- The paucity of data on free copper concentrations and phytoplankton limit conclusions that can be drawn
- Currently sampling in South Bay free copper concentrations using 4 analytical methods (Santa Cruz for Office of Naval Research)

Dr. Bruland – Question 2 (telephone interview)
- Would request additional monitoring of free copper concentrations and better characterization of phytoplankton populations

2.   Review Comments from Dave Hansen

From: Dhansen334@aol.com
Sent: Friday, March 31, 2000 3:56 AM
To: Tom.Grieb@tetratech.com
Subject: Re: San Francisco Bay TMDL

The article on the net still does not clear up the previous papers showing unique sensitivities of certain cyanobacteria. Only repeat toxicity tests of high quality can do that. It does indicate that even if certain of these cyanobacteria are sensitive, others seem to flourish in the Bay.

3.   Review Comments from Jonathan T. Phinney, Ph.D.

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April 10, 2000

To: Tom Grieb

From: Jonathan T. Phinney Ph.D.

RE: Review of Recent Information on the Occurrence of Cyanobacteria in Lower South San Francisco Bay

I have reviewed two recent articles sent by your office (Palenik and Flegel 1998 Findings from SFEI website); Ning et al (in press) in *Limnology and Oceanography*) and below is my response to your two questions regarding cyanobacteria abundance in San Francisco Bay. (Original questions are in italics).

1.   *The TMDL Workgroup would like to know if the results from the two studies would lead you to modify your original assessment regarding the toxicity of Copper to sensitive phytoplankton as well as your general conclusions regarding impairment?*

The two articles definitely demonstrate the consistent presence of the cyanobacteria in the San Francisco Estuary. In particular, Figure 2 from Ning et al (in press) records *Synechococcus* (a species of cyanobacteria) abundance in the extreme South Bay below the Dumbarton Bridge (50 km from the Central Bay) during July and August with a cell density of approximately 10^8 cells per liter. In addition, picocyanobacteria account for approximately 10 % of the total primary production in San Francisco Bay. While the authors caution that this figure may be an overestimate, it is consistent with other temperate estuaries.

It is during the summer when riverine and groundwater inputs are minimal to the bay and the dominant freshwater inputs are from the three municipal wastewater treatment facilities. Accordingly, it is during this period that the final effluent from the facilities will have its greatest effects on the ecosystem.

My original caution on Cu toxicity in South San Francisco Bay centered on the lack of data for effects to single cell organisms. In particular *Synechococcus* is considered the most susceptible organism to Cu toxicity to date (Brand et al. 1986. Reference listed in original document). The consistent presence of the bacteria in the South Bay removes the final caution that I have regarding Cu toxicity. Based on available evidence, I would reject the original null hypothesis that Cu and Ni impairment exists in the lower South San Francisco Bay.

2.   *In addition, if you were to modify your conclusions regarding impairment, how would you change your recommendations for follow-on studies?*

While these two studies demonstrate the consistent presence of *Synechococcus* in the South Bay, there is no data during wet weather events. Storm events may add pulses of Cu in a labile form species that is biologically available (i.e. not complexed by organic ligands). These pulses would likely originate from nonpoint sources such as land-based run-off, and may include combined sewer overflow systems (CSO's). (Note. I am not familiar with whether CSO's are present in the South Bay). Accompanying experiments could include dry and wet weather sampling and subsequent bioassay with sensitive cyanobacteria species as describe in my 9-24-99 assessment. If the results of toxicity tests are positive, then Cu speciation measurements would be a logical next step.

The lack of wet weather monitoring is problematic of most surveys, and EPA is now wrestling with the issue of wet weather standards. Any scientific study that incorporates wet weather events would be very relevant. I would strongly encourage the treatment facilities to go forward on such a study in the South Bay.

Appendix I
Cyanobacterial Populations in the San Francisco Bay

APPENDIX I
CYANOBACTERIAL POPULATIONS IN THE SAN FRANCISCO BAY

Brian Palenik[1] and A. Russ Flegal[2]

Introduction and Objectives

Anthropogenic inputs of metals to coastal environments have the potential to alter ecosystem productivity beginning with direct effects on phytoplankton. Metal inputs can also have the more subtle effect of changing phytoplankton species composition. Such a change then propagates through the ecosystem as grazers on particular phytoplankton groups are reduced or favored. A comprehensive review of these issues with specific reference to San Francisco Bay has recently been prepared (Tetra Tech 1999).

Marine cyanobacteria, in general, are thought to be particularly sensitive to copper toxicity based on lab studies (Brand, Sunda et al. 1986). In field studies high copper levels in small coastal bays have been correlated with the reduction in cyanobacteria of the genus *Synechococcus* (Moffet, Brand et al. 1997). In San Francisco Bay, cyanobacteria have been regarded as being "not commonly found" based on a review by Cloern (Cloern 1996) although this review was summarizing the phytoplankton populations of the spring bloom. Some data have suggested that cyanobacteria are present in San Francisco Bay (Murrell and Hollibaugh 1998), however we have little quantitative information on cyanobacterial abundance and its spatial and temporal variations. This information would be particularly important if cyanobacteria were regarded as indicator species for metal-impacted environments.

Cyanobacteria use proteins called phycobiliproteins to harvest light for photosynthesis. All cyanobacteria use the biliproteins phycocyanin and allophycocyanin for light harvesting. Some cyanobacteria also contain the biliprotein phycoerythrin. Cyanobacterial isolates without this protein generally appear green, but cyanobacterial isolates with this protein are red to brown colored. When examined with blue light excitation on an epifluorescence microscope or flow cytometer, cyanobacteria with phycoerythrin will be detected because phycoerythrin absorbs this blue light and fluoresces. Cyanobacteria without phycoerythrin are not easily detectable under these conditions.

A flow cytometer uses a laser for fluorescence excitation and hydrodynamic focusing of a sample to rapidly examine the fluorescence properties of individual cells. Analyzing cell counts with a flow cytometer can be much faster than using a microscope. The instrument has been used extensively to analyze *Synechococcus* and other cyanobacterial populations in marine environments (Olson, Chisholm et al. 1990). It has been used less often in analyzing coastal or estuarine systems. Large particles more common in natural coastal samples can clog the sampling system for example. We wanted to utilize the flow cytometer to see if we could detect cyanobacteria in San Francisco Bay and, if they were found, to analyze their spatial and temporal variation in the bay. Rapid analysis of cyanobacteria in samples might make their use as an indicator species more attractive for water quality monitoring.

Preliminary Results

We have examined the concentration of phycoerythrin-containing cyanobacteria in the San Francisco Bay ecosystem using flow cytometry analysis of samples from the February, April, and July Regional Monitoring Program cruises. Samples were fixed with glutaraldehyde and frozen for analysis back in the laboratory. Samples were thawed and filtered through a 100 μm screen to avoid large particles. A bead standard was added to all samples. The cell counts obtained by the flow cytometer were corrected to account for counting efficiency of the known bead standard.

In February and April 1999 the levels of phycoerythrin-containing cyanobacteria in the South Bay were at or near the detection level of the instrument while levels in the North Bay were easily detectable at around 1,000 to 6,000 cells/ml. In July 1999 however cell concentrations in the South Bay were up to 50,000 cells/ml, levels similar to those seen in Southern California coastal waters, while in the North Bay cell levels were similar to those seen in April.

A sample from the South Bay was not fixed with glutaraldehyde and, after shipment to the lab, subaliquots were filtered through 1.2 μm filters to enrich for cyanobacteria. After enrichment under white light conditions, the samples were examined and plated on agar plates of the same media. Colonies of cyanobacteria were isolated and regrown in the original media.

Enrichments from samples from the South Bay showed the presence of at least three different cyanobacterial types – two likely related to *Synechococcus* and one resembling *Synechocystis* in that it forms small rafts of cells. For the former, one *Synechococcus* type isolate is green (likely lacking phycoerythrin) while one type is red (contains phycoerythrin). Thus although the South Bay shows high copper and other metal levels it seems to support the growth of a diverse cyanobacterial population. The biochemical adaptations of these cyanobacteria to the metal levels in their environment remain unknown.

Future Directions

Cyanobacteria are present in San Francisco Bay and interestingly in the South Bay where metal levels are relatively high. Their presence could be explained by:

1) Copper levels are not toxic because of the presence of other metals such as manganese that ameliorate the copper toxicity.

2) The cyanobacterial species found in the South Bay are less sensitive to metals than the species studied by Brand (Brand, Sunda et al. 1986). If they are less sensitive, what adaptations do they posess that are absent from the strains studied by Brand? Are these adaptations characteristic of particular cyanobacterial "species"? If so, can one define cyanobacterial species that might be indicators for metal impacted environments?

These questions can possibly be answered using the isolates we have brought into culture by studying their sensitivity to copper at different copper/manganese ratios for example. We can also begin to compare what proteins they express ar high copper levels compared to strains used by Brand.

The flow cytometer approach using a 488 nm laser only readily analyzes cyanobacteria with phycoerythrin, but cyanobacteria without phycoerythrin were found in our enrichments. In the future we would also like to compare an epifluorescence microscope approach for counting cyanobacteria with the flow cytometer. In this way would we understand what percentage of cyanobacteria are of the phycoerythrin-containing type and what percentage have pigments similar to the phycoerythrin lacking (green-colored) *Synechococcus* and *Synechocystis* type cultures.

Brian Palenik[1] Scripps Institution of Oceanography, University of California, San Diego. La Jolla, California

Russ Flegal[2] Environmental Toxicology. University of California, Santa Cruz. Santa Cruz, California.

References

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Spatial and temporal variability of picocyanobacteria *Synechococcus* sp. in San Francisco Bay

Abstract—We collected samples monthly, from April to August 1998, to measure the abundance of autotrophic picoplankton in San Francisco Bay. Samples taken along a 160-km transect showed that picocyanobacteria (*Synechococcus* sp.) was a persistent component of the San Francisco Bay phytoplankton in all the estuarine habitats, from freshwater to seawater and during all months of the spring–summer transition. Abundance ranged from 4.6×10^6 to 5.2×10^8 cells L^{-1}, with peak abundance during the spring bloom (April and May) and during July with a persistent spatial pattern of smallest abundance near the coastal ocean and highest abundance in the landward domains of the estuary. The picocyanobacterial component (as estimated percentage of chlorophyll *a* concentration) was, on average, 15% of total phytoplankton biomass during the summer–autumn nonbloom periods and only 2% of chlorophyll biomass during the spring bloom. This result is consistent with the emerging concept of a gradient of increasing importance of picocyanobacteria along the gradient of decreasing nutrient concentrations from estuaries to the open ocean.

For two decades now biological oceanographers and limnologists have explicitly recognized the importance of micron-sized phytoplankton (picoplankton) as components of the autotrophic communities of pelagic systems. The picoplankton, predominantly coccoid cyanobacteria (*Synechococcus* sp., Johnson and Sieburth 1979; Waterbury et al. 1979), can be major contributors of phytoplankton biomass and production in the oceans (Joint 1986; Olson et al. 1990) and lakes (Stockner 1988). The size distribution of the phytoplankton, and in particular the partitioning between picoplankton and larger cells, is a fundamental aspect of pelagic systems that (a) reflects the source and cycling of nutrients, and (b) influences the pathways through which production is transferred to consumers. In general, we associate the picoplankton with low-nutrient conditions where primary production is sustained by regenerated nutrients (Chisholm 1992); picoplankton production is first transferred to consumers by protozoan grazing since most metazoans cannot effectively capture micron-sized algal cells (Tamigneaux et al. 1995; Vaquer et al. 1996). On the other hand, we associate the larger phytoplankton (especially fast-growing diatoms) with high-nutrient conditions where primary production is sustained by inputs of new nutrients; trophic transfer of large-cell production begins with metazoan grazing, and some fraction of this production is exported by sinking.

The distinction between picoplankton regenerating systems and large-cell new-production systems results, in part, from the competitive advantage of small cell size under conditions of resource limitation (Raven 1986; Riegman et al. 1993). This competitive advantage disappears under high-nutrient conditions because the picoplankton population growth is tightly regulated by the fast-growing protozoan consumers (Ning and Vaulot 1992), whereas the larger cells have (at least temporary) refuge from predation by the slower-growing metazoan grazers (Malone 1992; Riegman et al.

1993). Therefore, inputs of new nutrients tend to promote net population growth and biomass accumulation of larger cells (Malone 1992). As a result of these differences in size-related growth and grazing rates, the picoplankton component of production is highest in the oligotrophic regions of the ocean (Joint 1986; Chisholm 1992). The picoplankton component also increases in regions (Joint 1986; Ning et al. 1996), and during seasons (Malone 1992; Li 1998) of high water temperature because the picoplankton have a stronger growth response to temperature variability than the larger eucaryotic cells (Andersson et al. 1994). So, the size-related aspects of pelagic primary production and trophic transfer seem to be determined largely by the nutrient-temperature regime (Malone 1992). This principle would suggest that estuaries, which have continual inputs of exogenous nutrients from their watersheds, might act as new-production systems that tend to favor production of large cells (Riegman et al. 1993). In fact, Iriarte and Purdie (1994) have proposed that phytoplankton size distribution changes along the eutrophication gradient from the land margin to the open ocean, with the picoplankton contribution >50% offshore, ~20% in the coastal ocean, and <10% in estuaries. The few studies of estuarine picoplankton ecology are generally consistent with this hypothesis, although there are exceptions such as the Thau Lagoon (France) where the picoplankton contribute nearly 40% of primary production (Vaquer et al. 1996). This special case might be explained by the unusual intensity of (size-selective) suspension feeding by oysters reared in this lagoon. Therefore, the balance between picoplankton and larger-cell production in estuaries might be determined by a combination of nutrient/temperature-driven differences in growth rate and the strength of grazing by benthic/epibenthic suspension feeders that typically select larger cells.

San Francisco Bay as a gradient of estuarine habitats—Here, we present results of a study designed to measure the abundance of the picocyanobacteria (*Synechococcus*) in San Francisco Bay as an example of a nutrient-rich, temperate-zone estuary in which phytoplankton dynamics are influenced by the benthic suspension feeders. San Francisco Bay has been a site of sustained estuarine research for three decades, and one focus has been to identify the patterns and mechanisms of estuarine phytoplankton dynamics measured as spatial-temporal variability of chlorophyll biomass and primary production (Cloern 1996). Although studies have been conducted to partition biomass and production among algal size classes (Cole et al. 1986), there has been no study yet to measure the picocyanobacterial component of the phytoplankton in this estuary. San Francisco Bay is a useful site for general estuarine research because it comprises geographic subsystems that provide large gradients in the physical, chemical, and biological components of habitat that influence phytoplankton population dynamics, including those components thought to shape the composition of phyto-
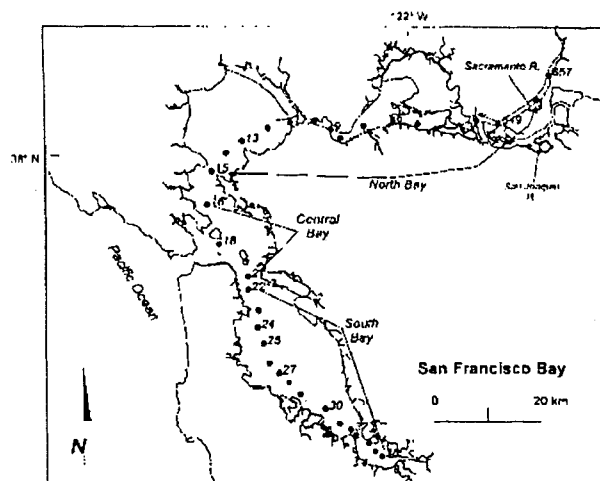
Fig. 1. Map of the San Francisco Bay estuarine system, showing locations of hydrographic sampling (CTD profiles, nutrients, chlorophyll) and sampling for cyanobacteria abundance (numbered circles).

plankton communities. The northern reach (North Bay, Fig. 1) is a partially stratified estuary of the Sacramento-San Joaquin Rivers, with longitudinal gradients of salinity, turbidity from suspended sediments, and dissolved inorganic nutrients. The North Bay is strongly influenced by seasonal fluctuations in river discharge, which varies from (monthly mean) flows of ~2,000 m³ s⁻¹ during winter–spring to summer–autumn minima of ~100–200 m³ s⁻¹. By contrast, the South Bay is a semienclosed marine lagoon system that is influenced by riverine discharge only during the high-flow season, but persistently influenced by nutrient inputs from the densely populated local watershed (Hager and Schemel 1996). Between these distinct subsystems is the Central Bay (Fig. 1), a deep region 'where water masses from the North Bay, South Bay, and the coastal Pacific Ocean are mixed by tidal currents.

Past studies of bulk quantities (Chl a, primary production) have shown how phytoplankton dynamics in San Francisco Bay are influenced by the spatial gradients and seasonal variability of the bottom-up, top-down, and hydrodynamic processes that control the balance between phytoplankton production, consumption, and transport in estuaries. In particular, nutrient (dissolved inorganic N, P, Si) concentrations are usually above levels that limit phytoplankton growth (Cloern 1999); light limitation is a strong controlling force on phytoplankton growth rates, and spatial gradients of primary production closely parallel the river-ocean gradient of suspended sediments and light availability (Cloern 1996); top-down control is dominated by the consumption of phytoplankton cells by benthic suspension feeders, which balances primary production in the landward regions of the North Bay (Alpine and Cloern 1992) and exerts a strong seasonal control on phytoplankton dynamics in South Bay; key physical processes include tidally-driven vertical mixing and salinity stratification as these influence the growth-graz-

ing balance (Lucas et al. 1998). The phytoplankton community as a whole, responds to changing physical dynamics (river flow, tidal mixing, stratification) in the South Bay during spring, when a bloom occurs each year. On this foundation of past study, we ask here the first-order questions about picocyanobacterial ecology: What is their contribution to the total community biomass (and potential production), and how does this contribution vary (a) spatially along the large habitat gradients? and (b) seasonally in response to changes in the riverine and tidal forcings that are so prominent in estuaries?

Methods—We conducted monthly sampling cruises from April through August 1998, to map the spatial distributions of habitat descriptors and picocyanobacteria abundance along a 160-km transect between the North Bay, Central Bay, and South San Francisco Bay. At each sampling location (Fig. 1), we measured vertical profiles of salinity and temperature (Sea-Bird Electronics SBE-9/11 CTD), chlorophyll fluorescence (Sea Tech fluorometer), and turbidity (D&A Instruments OBS sensor). Near-surface (~2 m) water samples were collected at some of these stations with a 5-liter Niskin bottle, and aliquots were analyzed for: Total Chl a (samples collected onto A/E glass fiber filters, extracted in 90% acetone, and concentration determined spectrophotometrically; Lorenzen 1967); suspended sediment concentration (measuring the dry weight of seston collected onto preweighed 0.4 μm Nuclepore filters); and dissolved inorganic nitrogen and phosphorus (using filtrates passing 0.4 μm Nuclepore filters, and analyzed with a Technicon Autoanalyzer II). The discrete measures of Chl a and suspended sediment concentration were used to calibrate the fluorometer and OBS sensor each cruise. Complete results of this sampling program are available over the Internet. Aliquots of some water samples were preserved in acidified Lugol's solution and later examined under light microscope to identify and count the eucaryotic phytoplankton.

Water samples for cyanobacteria enumeration were fixed in 1% paraformaldehyde and stored in polyethylene bottles. Sample bottles were held at room temperature for 10 min and then frozen immediately in liquid nitrogen and stored at −80°C. Samples for microscopic determinations of autotrophic picocyanobacteria were filtered onto black polycarbonate membrane filters with a pore size of 0.2 μm, and enumerated under an Olympus BH-2 epifluorescence microscope equipped with a 100 W mercury lamp and Olympus G filter set, or with a Nikon ECLIPSE E800 epifluorescence microscope equipped with a 100 W mercury lamp and Nikon EF-4 FITC/TRITC (F-R) 25 mm dual filter cube. The G filter set was supplemented with an EO530 excitation filter and O590 barrier filter (a long pass filter) to produce narrow-band green excitation around 530 nm. With this combination of excitation and emission filters, phycoerythrin-containing *Synechococcus* fluoresced bright orange-yellow, its emission wavelength around 590–630 nm (Hofstraat et al. 1991). Slides were counted using Plan Apo ×40 objectives with the Olympus BH-2 and ×60 or ×100 oil-immersion objectives with the Nikon E-800 microscope with ×15 oculars. For each sample, a minimum of 10 reticule fields with at least 400 cells were counted. For a few samples that had very low

abundance, cell counts were accumulated over 20 reticule fields. For our procedures, counting error that included intercalibration between the two epifluorescence microscopes typically averaged 5% (C.V.). Dimensions (diameter of coccoid cells, diameter and length of rod-shaped cells) were recorded for all enumerated cells.

Results and discussion—This study was designed to follow changes during the spring–summer transition when river discharge recedes, water temperature increases, and chlorophyll biomass declines following the spring bloom. The top panels of Fig. 2 show the changing spatial distributions of near-surface salinity and temperature during the five sampling cruises. The first (April) sampling occurred after months of high river flow and diluted salinities throughout San Francisco Bay. Near-surface salinity ranged between about 10–17 psu in South Bay, 15 psu in Central Bay, and from 15–0 along the North Bay. As river flow receded, salinities progressively increased and reached August maxima of 20–25 in South Bay, ~30 in Central Bay, and from 20–0 along the North Bay (Fig. 2). These changing salinity distributions reflect the changing balance between the riverine input of fresh water and the physical processes that drive horizontal advection and mixing along the estuary and exchanges with the coastal ocean. Shapes of the salinity profiles along the 160-km transect show that these balances were different for the South Bay, Central Bay, and North Bay. Together with the temperature profiles, these confirm the distinct character of the South Bay as a marine-brackish lagoon, the North Bay as a river-dominated estuary, and the Central Bay as an estuarine zone having a strong influence of mixing with the coastal ocean. Surface temperatures were fairly uniform in April (~13–14°C) and May (~15–16°C), but there were large horizontal temperature gradients in the summer months when water temperature increased rapidly in the landward domains of both the South Bay and North Bay. For example, during July we measured surface temperature of 13.7°C in the Central Bay and 23.6°C in the Sacramento River and upper estuary (Fig. 2).

The horizontal distributions of suspended particles, both sediments and phytoplankton (as chlorophyll biomass), were consistent with the notion of distinct subdomains within the San Francisco Bay system (Fig. 2). Spatial distributions of suspended particulate matter (SPM) along the North Bay–Central Bay showed a localized turbidity maximum that was seaward and intense in April, when near-surface SPM concentration was over 100 mg L⁻¹. This turbidity maximum became displaced landward as river flow receded during summer. SPM concentrations were consistently low in the Central Bay, reflecting the large distance from the riverine source of sediments and rapid exchange with the coastal ocean. In the lagoonal South Bay, SPM concentrations were highly variable, especially in April when high concentrations (>250 mg L⁻¹) were measured at the landward extreme. Chlorophyll distributions showed an intense spring phytoplankton bloom in April 1998, with elevated concentrations of Chl *a* throughout San Francisco Bay. The highest Chl *a* concentrations occurred in the landward reach of the South Bay, with peaks >160 μg L⁻¹ and progressive dilution of chlorophyll toward the Central Bay. A second local maxi-

mum occurred in the landward reach of the North Bay, coincident with the turbidity maximum, where near-surface Chl *a* was 45 μg L⁻¹. This same feature was observed in May (Fig. 2), but with reduced Chl *a* concentrations (13 μg L⁻¹). The spring bloom was a period of high abundance of several species of coastal diatoms (*Skeletonema costatum, Chaetoceros debilis, C. subtilis, C. gracilis*), phytoflagellates (*Teleaulax amphioxeia, Rhodomonas salina, Pyramimonas orientalis, Plagioselmis prolonga*), chlorophytes (*Chlorella marina, Nannochloris atomus*), and the dinoflagellate *Heterocapsa rotundata*. We measured low Chl *a* concentrations throughout the estuary during the June, July, and August cruises, consistent with past observations of low phytoplankton biomass during summer.

Seasonal changes in dissolved inorganic nitrogen and phosphorus were also similar to those observed in other years, with highest concentrations of dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) in the landward reaches of the South Bay, reflecting the large local inputs from the urban watershed (Hager and Schemel 1996). Nutrient concentrations were more homogeneous along the North Bay–Central Bay transect. In all three subregions, the DIP concentration was always above 0.75 μM, typically between 1–2 μM, and never at levels that would severely limit phytoplankton growth. DIN was also usually well above rate-limiting concentrations, except for measurements made in the seaward reach of the South Bay in April (Fig. 2). From previous observations, we infer that this localized depletion of DIN was ephemeral; by the May cruise, DIN concentrations in this region had already recovered to 10 μM. Therefore, while DIN depletion may have played a role in limiting the size of the spring bloom in South Bay, observations in 1998 were consistent with the idea that San Francisco Bay is a nutrient-rich estuary and that nutrient limitation plays only a minor role in regulating phytoplankton growth rate.

These results show that the spatial-temporal variability encountered during the study encompassed much of the habitat variability found in temperate-zone estuaries: salinity ranged from 0 to 30 psu; temperature ranged from 12.9 to 24.0°C; near-surface SPM concentrations ranged from 2 to >250 mg L⁻¹; phytoplankton biomass ranged from nonbloom conditions of 1.3 μg L⁻¹ Chl *a* to a massive bloom with Chl *a* >160 μg L⁻¹; and a short-term, localized event of DIN depletion occurred against a background of high DIN and DIP concentrations. Across this broad range of habitat conditions, we measured changes in cyanobacterial *Synechococcus* abundance that varied two orders of magnitude, from a minimum of 4.6 × 10⁶ cells L⁻¹ in Central Bay (June) to a maximum of 5.2 × 10⁸ cells L⁻¹ in the South Bay (April, Fig. 2). The autotrophic picocyanobacteria we observed was phycoerythrinbilin rich *Synechococcus*, with cell diameters ranging from 0.5–1.5 μm. *Synechococcus* was the dominant component of photosynthetic cyanobacteria, and its abundance normally comprised more than 95% of total abundance of photosynthetic picoplankton; picoeucaryotes were scarce in nearly all the samples observed. *Synechococcus* abundance ranged from 7.0 × 10⁶ to 5.2 × 10⁸ cells L⁻¹ (mean = 1.3 × 10⁸) in April; 1.8 × 10⁷ to 3.4 × 10⁸ cells L⁻¹ (mean = 1.6 × 10⁸) in May; 4.6 × 10⁶ to 6.3 × 10⁷
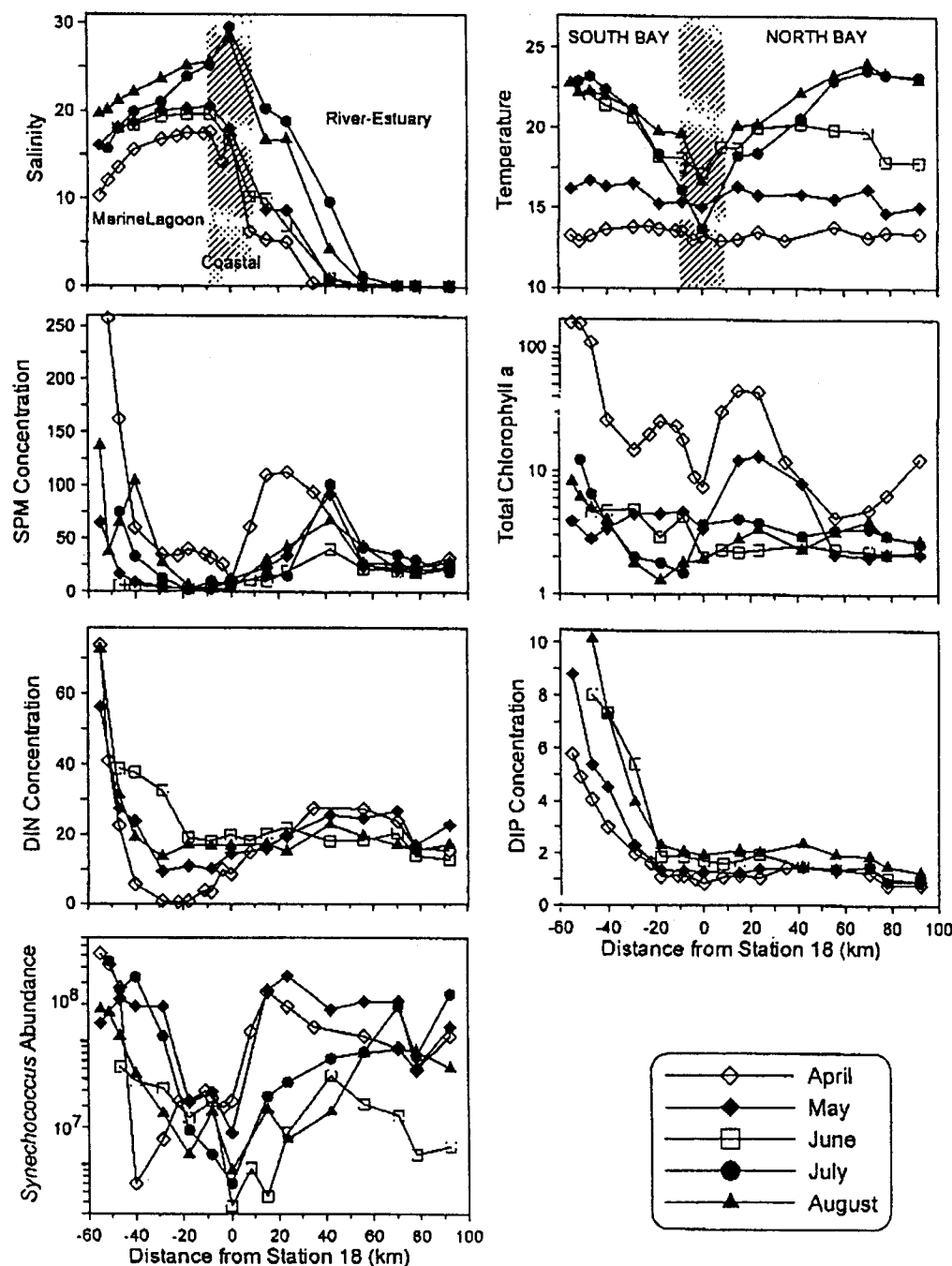
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Fig. 2. Spatial distributions of habitat descriptors and *Synechococcus* abundance along the 160-km longitudinal transect in San Francisco Bay, comparing results from monthly sampling between April and August 1998. Distances are measured from Sta 18 in the Central Bay, with negative values in the South Bay and positive values in the North Bay. Distributions of salinity and temperature (upper panels) illustrate the marine/brackish character of the lagoonal South Bay, strong coastal influence in the Central Bay, and the river-estuary continuum of the North Bay. Panels below show, in sequence, the changing spatial distributions of SPM concentration (mg L^{-1}), total Chl a concentration (μg L^{-1}), dissolved inorganic nitrogen and phosphorus (μM), and *Synechococcus* abundance (cells L^{-1}).

Notes

Table 1. Mean (±SD) values of near-surface temperature, salinity, SPM, Chl a, and abundance of picocyanobacteria, for the three subregions of San Francisco Bay. Sample number (N) is the number of samples analyzed for picocyanobacteria cell abundance in each region and for each month.

Month 1998	Bay*	Temp (°C)	Salinity (psu)	SPM (mg L⁻¹)	Chl a (μg L⁻¹)	Cell abundance (10⁷ cells L⁻¹)	N
Apr	North Bay	13.4 ± 0.3	1.6 ± 2.4	59.9 ± 43.4	18.5 ± 18.3	13.4 ± 6.5	7
	Central Bay	13.2 ± 0.3	13.8 ± 5.3	32.3 ± 21.3	16.2 ± 10.4	5.4 ± 4.4	4
	South Bay	13.5 ± 0.3	15.1 ± 2.8	139.4 ± 163.8	67.1 ± 64.3	16.7 ± 20.7	8
May	North Bay	15.7 ± 0.6	2.6 ± 4.1	36.1 ± 24.9	6.0 ± 5.1	19.9 ± 9.0	7
	Central Bay	15.2 ± 0.2	19.3 ± 1.8	7.0 ± 7.1	4.1 ± 0.9	2.9 ± 1.5	2
	South Bay	16.2 ± 0.6	18.6 ± 1.7	19.8 ± 25.8	3.8 ± 0.7	15.4 ± 7.4	5
Jun	North Bay	19.2 ± 1.0	2.6 ± 4.1	22.1 ± 9.0	2.3 ± 0.1	2.3 ± 1.6	7
	Central Bay	18.1 ± 0.8	15.5 ± 4.8	8.0 ± 3.6	2.9 ± 1.3	1.6 ± 1.5	3
	South Bay	20.6 ± 1.7	18.9 ± 0.7	4.8 ± 1.5	4.3 ± 0.9	4.4 ± 1.6	4
Jul	North Bay	21.5 ± 2.4	7.2 ± 9.1	36.9 ± 30.1	3.3 ± 0.5	10.6 ± 7.7	7
	Central Bay	14.9 ± 1.7	27.4 ± 3.0	8.0 ± 2.8	2.6 ± 1.6	1.0 ± 0.4	2
	South Bay	21.6 ± 2.0	19.7 ± 3.1	107.8 ± 174.5	5.3 ± 4.4	23.4 ± 17.2	5
Aug	North Bay	22.3 ± 1.6	5.5 ± 7.8	34.7 ± 17.4	3.0 ± 0.5	5.5 ± 3.0	7
	Central Bay	18.2 ± 2.1	26.9 ± 1.9	3.0 ± 0.0	1.9 ± 0.1	1.8 ± 1.3	2
	South Bay	21.7 ± 1.1	21.9 ± 2.1	62.7 ± 49.8	4.4 ± 2.6	9.2 ± 7.3	6

* North Bay: Sta 15, 13, 11, 9, 6, 3, 649, 657; Central Bay: Sta 21, 20, 18, 16; South Bay: Sta 36, 34, 32, 30, 27, 25, 24, 22.

cells L⁻¹ (mean = 2.7 × 10⁷) in June; 7.0 ×₁10⁶ to 4.5 × 10⁸ cells L⁻¹ (mean = 1.4 × 10⁸) in July; and 8.9 × 10⁶ to 1.8 × 10⁸ cells L⁻¹ (mean = 6.5 × 10⁷) in August (Table 1). The average values of picocyanobacteria abundance and the related environmental parameters in the three subsystems of San Francisco Bay are summarized in Table 1.

We observed picocyanobacteria in all samples collected during this study, at abundances >4.6 × 10⁶ cells L⁻¹, so *Synechococcus* is a persistent component of the San Francisco Bay phytoplankton. We observed large spatial variability of *Synechococcus* abundance, with a consistent spatial pattern of minimum abundances in the Central Bay and highest abundances in the landward domains of both the South Bay and North Bay (Fig. 2). This spatial pattern suggests that the picocyanobacterial component is sustained by population growth within the bay system, where the balance between production and losses is more positive in the estuarine-lagoonal domains than in the coastal-dominated marine domain. One potential explanation is that the spatial gradient of picocyanobacteria abundance is produced, in part, by the spatial gradient of water temperature as a regulator of *Synechococcus* growth rate (Joint 1986; Ning and Vaulot 1992; Andersson et al. 1994). The horizontal distributions of picocyanobacteria closely paralleled the horizontal temperature

gradients (Fig. 2). Significant positive correlations between cyanobacteria abundance and temperature were present, especially in June, July, and August when the spatial gradients of temperature were pronounced. Other factors, such as salinity, SPM, and nutrient concentrations, were only weakly correlated with the abundance of cyanobacteria (Table 2).

Li's (1998) intensive study of *Synechococcus* abundance in Bedford Basin showed a consistent seasonal pattern of variability characterized by low winter abundances and maximum abundances in September. Our results did not show such a clear pattern of monthly variability within the San Francisco Bay system (Fig. 2). Rather, we observed high *Synechococcus* abundances in April (mean = 1.3 × 10⁸ cells L⁻¹; Table 1) in association with the spring bloom, and in May (mean = 1.6 × 10⁸ cells L⁻¹) when *Synechococcus* cells were mostly small (0.5–0.8 μm diameter), but also in July (mean = 1.4 × 10⁸ cells L⁻¹) when total chlorophyll biomass was low. Smallest abundances were observed in June (mean = 2.7 × 10⁷ cells L⁻¹). High abundance in April suggests that the picocyanobacteria population responded to the changing physical dynamics of the estuary during spring, when the populations of diatoms and phytoflagellates also grew very rapidly; high abundance in May with smaller cell size reflected *Synechococcus* fast growth and less strong

Table 2. Correlation coefficients (r) between picocyanobacteria abundance and temperature, salinity, SPM concentration, and Chl a. Correlations are based on near-surface measurements made at 14 to 19 sites in San Francisco Bay each month, from April to August 1998.

Month 1998	Temperature r	P	Salinity r	P	SPM r	P	Chl a r	P
Apr	0.55	<0.02	0.45	<0.05	0.67	<0.01	0.68	<0.001
May	0.70	<0.01	0.36	>0.10	0.36	<0.10	0.36	<0.10
Jun	0.76	<0.001	0.15	>0.10	0.06	>0.10	0.69	<0.01
Jul	0.91	<0.001	0.53	<0.05	0.48	<0.10	0.49	<0.10
Aug	0.81	<0.001	0.39	<0.10	0.51	<0.05	0.80	<0.001

Notes

Table 3. Estimated biomass of picocyanobacteria as chlorophyll concentration Pico-Chl *a* (μg L⁻¹) and as percentage of total Chl *a* biomass (% total).

Month 1998	Bay*	Pico-Chl *a* (μg L⁻¹)		Pico-Chl *a* (% Total)	
		Range	Mean (±SD)	Range	Mean (±SD)
Apr	North Bay	0.46–1.95	1.04 ± 0.51	3.4–20.5	9.3 ± 6.1
	Central Bay	0.23–0.94	0.43 ± 0.34	1.5–3.5	2.7 ± 0.9
	South Bay	0.09–4.06	1.32 ± 1.60	0.4–2.5	1.6 ± 0.6
May	North Bay	0.25–0.58	0.41 ± 0.12	3.6–25.1	12.0 ± 8.5
	Central Bay	0.14–0.30	0.22 ± 0.11	4.1–6.5	5.3 ± 1.7
	South Bay	0.25–0.89	0.65 ± 0.25	5.6–31.9	18.4 ± 9.8
Jun	North Bay	0.07–0.41	0.18 ± 0.12	3.4–16.6	7.8 ± 4.6
	Central Bay	0.06–0.44	0.21 ± 0.20	3.1–10.3	6.3 ± 3.7
	South Bay	0.32–0.85	0.60 ± 0.22	11.1–18.4	13.6 ± 3.4
Jul	North Bay	0.28–0.97	0.47 ± 0.28	6.9–37.5	15.3 ± 11.0
	Central Bay	0.09–0.16	0.13 ± 0.05	2.5–10.8	6.7 ± 5.9
	South Bay	0.15–1.83	0.97 ± 0.68	8.2–35.3	19.4 ± 10.2
Aug	North Bay	0.12–0.68	0.43 ± 0.24	3.7–22.4	13.9 ± 7.0
	Central Bay	0.12–0.36	0.24 ± 0.17	6.3–20.2	13.3 ± 9.8
	South Bay	0.16–1.41	0.73 ± 0.55	10.7–21.8	15.1 ± 4.3

* North Bay: Sta 15, 13, 11, 9, 6, 3, 649, 657; Central Bay: Sta 21, 20, 18, 16; South Bay: Sta 36, 34, 32, 30, 27, 25, 24, 22.

grazing by heterotrophic nanoplankton. High abundances in July are consistent with observations in other temperate-boreal estuaries (e.g., Malone 1992; Lewitus et al. 1998) where peak annual abundances are observed during some summer months. The low abundances in June and August suggest that the balance between cyanobacteria growth and grazing loss fluctuates at the monthly time scale. We know, from past studies (Ambler et al. 1985), that the population dynamics of protistan grazers, such as tintinnid ciliates, are characterized by high-amplitude fluctuations during spring–summer. We did not measure grazing loss rates of cyanobacteria in our study, but such measurements in other systems have shown tight regulation of cyanobacteria abundance by protistan grazing. For example, grazing rates by heterotrophic nanoplankton were high enough to balance the growth rates of picocyanobacteria in the English Channel (Ning and Vaulot 1992) and in North Inlet during summer (Lewitus et al. 1998).

In order to assess the potential ecological significance of cyanobacteria in San Francisco Bay, we transformed *Synechococcus* cell abundances into estimated chlorophyll biomass, and then compared these values to our measures of total chlorophyll concentration. Individual *Synechococcus* cells displayed various shapes, such as spheres or rods, but most (>80%) were coccoid cells in the size range of 0.5–1.4 μm diameter (the commonest was 0.8 to 1.2 μm diameter). Biovolume was calculated using the formulas $4/3$ (πr^3) for coccoid and $\pi r^2 h$ for rod-shaped cells. Cell volumes were transformed to carbon biomass, using the conversion factor 470 fg C μm⁻³ (Verity et al. 1992), corresponding to a carbon mass of 250 fg C for coccoid cells of diameter 1 μm. For transforming cell carbon biomass to Chl *a*, we used the conversion factor 32 g C g Chl *a*⁻¹ (Takahashi et al. 1985). These kinds of conversions yield estimates that are highly uncertain because of the large variability in the size and carbon and chlorophyll contents of *Synechococcus* cells

(e.g., Malone 1980). Estimated values of picocyanobacterial chlorophyll are summarized in Table 3.

The estimated picocyanobacterial component of Chl *a* biomass ranged from <1% to 38% in San Francisco Bay and, consistent with all similar assessments (e.g., Chisholm 1992; Iriarte and Purdie 1994), there was a strong inverse relation between the picocyanobacterial fraction (as percentage of total chlorophyll) and the total chlorophyll biomass (Fig. 3). This observation is also consistent with the notion that the picocyanobacterial component of biomass becomes significant during periods of low phytoplankton biomass, but this contribution is relatively small during bloom events when the biomass of larger eucaryotic cells grows explosively. We partitioned the full data set into bins corresponding to conditions of high phytoplankton biomass ("blooms") and low biomass, and then plotted picocyanobacterial biomass (as estimated Chl *a* concentration) against total Chl *a* concentration for each condition (Fig. 3, insets). Linear regressions were significant ($P < 0.01$), and the best fits were obtained when we defined the high-chlorophyll condition as events when total Chl $a > 7$ μg L⁻¹. The slopes of the two regression equations indicated that the mean picocyanobacterial contribution to total chlorophyll biomass was ~15% during nonbloom conditions but only 2% when Chl *a* exceeded 7 μg L⁻¹. The overall mean picocyanobacterial Chl *a* concentration was 0.61 μg liter⁻¹, accounting for an estimated mean 11% of the total measured Chl *a* concentration.

We have not measured directly the contribution of the picocyanobacteria to total phytoplankton primary production in San Francisco Bay, but during a yearlong study of size-fractionated primary production at six sites, Cole et al. (1986) demonstrated that the <5 μm fraction contributed from 6% to 28% of total annual primary production. Moreover, Cole et al. showed that the chlorophyll-specific carbon assimilation rates of the <5 μm, 5–22 μm, and >22 μm components of the phytoplankton were not significantly dif-
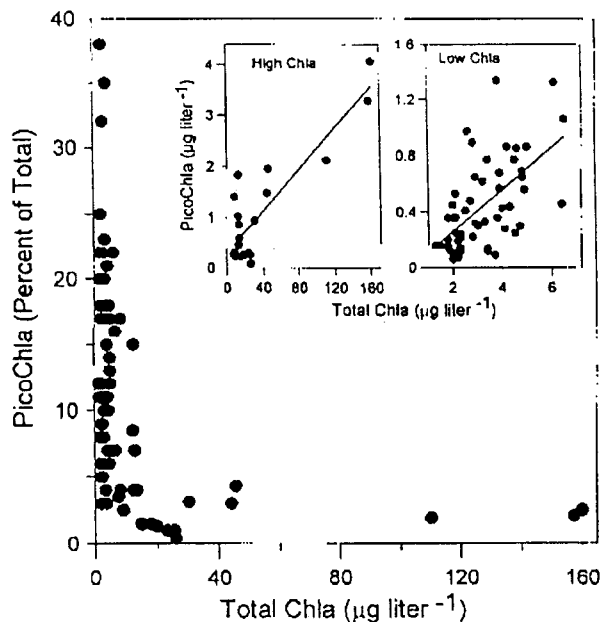
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Fig. 3. Picocyanobacteria biomass (as estimated percentage of Chl *a* concentration) vs. total phytoplankton biomass (as total Chl *a*), for the 76 samples collected in San Francisco Bay between April and August 1998. Upper insets show the correlations between picocyanobacteria biomass (as estimated Chl *a* concentration) and total Chl *a*. The data were partitioned into conditions of high Chl *a* (>7 μg L^{-1}) and low Chl *a* (<7 μg L^{-1}). Both regression lines were significant. High chlorophyll: $y = 0.31 + 0.02x$ ($r = 0.87$, $n = 21$); Low chlorophyll: $y = 0.04 + 0.15x$ ($r = 0.59$, $n = 55$).

ferent. This result implies that the contribution of each phytoplankton size class to total primary production scales directly with its contribution to total biomass. If this generality extends to the smallest (pico) size class, then the results presented here suggest that the picocyanobacteria might not contribute more than about 10% of the total primary production in San Francisco Bay. This figure might be an overestimate because our study of biomass was conducted during the warmest months when the picocyanobacterial contribution is expected to be greatest.

These conclusions are consistent with the hypothesis of Iriarte and Purdie (1994) that the picoplankton contribute about 10% of primary production in nutrient-rich estuaries. Our results are also consistent with the hypothesis that, since picoplankton abundance is tightly regulated by fast-growing protistan grazers, the picoplankton component of biomass is relatively stable and oscillates around a steady mode as a "dynamic equilibrium" (Fogg 1991). We estimate that the picocyanobacteria never reached biomass greater than 4.1 μg L^{-1} Chl *a*. On the other hand, total phytoplankton biomass reached 160 μg Chl L^{-1} when the abundance of larger eucaryotic cells (diatoms, phytoflagellates) grew rapidly during the spring bloom. Therefore, San Francisco Bay does appear to function primarily as a new-production system in which nutrient concentrations are (almost always) above those that

give selective advantage to small cells. There appear to be seasonal shifts in the relative importance of the new-production and regenerating systems, with small increases in the relative cyanobacterial component during the spring–summer transition. However, changes during this spring–summer transition are much smaller in San Francisco Bay than they are in other nutrient-rich estuaries such as Chesapeake Bay (Malone et al. 1991), the St. Lawrence estuary (Baie des Chaleurs, Tamigneaux et al. 1995), and the Baltic Sea (Uitto et al. 1997). Compared to these estuarine systems, San Francisco Bay is tidally energetic and subject to rapid turbulent mixing—a physical condition that precludes nutrient depletion of surface waters during summer (Fig. 2). Comparison among these ecosystems suggests that the relative importance of the picoplankton-selective regenerating state and the large-cell-selective new-production state (like many other aspects of pelagic dynamics) is strongly dependent upon physical dynamics, including processes that control the intensity of turbulent mixing and density stratification as these influence the relative importance of regenerated and exogenous sources of nutrients to estuarine phytoplankton.

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